JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXXII WASHINGTON, D. C., JANUARY 15, 1926 NO. 2

RINGSPOT OF CRUCIFERS CAUSED BY MYCOSPHAERELLA BRASSICICOLA (FR.) LINDAU [1]

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INTRODUCTION

Large quantities of cauliflower (including broccoli), cabbage, Brussels sprouts, and related cruciferous crops are grown each winter in California. Of these, cauliflower is by far the most important both from the standpoint of the quantity grown and the financial returns to the grower. The large producing centers are in Los Angeles, San Francisco, San Mateo, Alameda, and Sacramento Counties. In certain sections, principally in San Mateo and San Francisco Counties, crucifers are grown throughout the year, most of the production being from December to April, inclusive, while in most of the other counties these crops are not grown to any extent except during the winter season. Likewise, in certain sections, such as the west coast of San Francisco Bay, cruciferous crops are grown so intensively that they occupy a major portion of the tillable land during about half of the year. In other parts of the State the fields planted to these crops are more isolated. As Colma is the shipping center for most of the cauliflower grown on the west side of San Francisco Bay, this territory will be designated as the Colma section. The work forming the basis of this paper was done for the most part in the Colma section; hence, unless otherwise stated, all discussions have reference to it.

HOSTS

Practically all of the crucifers grown in the Colma section are more or less seriously affected with a leafspot. This disease has been found on cauliflower, white broccoli, black broccoli, rutabaga, Brussels sprouts, kale, cabbage, red cabbage, and Savoy cabbage, but not on radish or the white turnip. All strains and varieties of the plants on which the disease has been found are susceptible. Practically all of these crops are of locally developed strains produced from home-grown seed. In general, the order of susceptibility seems to be as follows: White broccoli, black broccoli, cauliflower, Brussels sprouts, cabbage, yellow turnip, and kale. Although there is no striking difference in the susceptibility of the first five hosts named, these are no doubt most seriously injured.

[1] Received for publication May 18, 1925; issued February, 1926.

[2] The writer wishes to thank R. E. Smith and William T. Horne of the Department of Plant Pathology, University of California, who placed at his disposal the laboratory, garden, and greenhouse facilities used in this work.

HISTORY AND GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

The disease under discussion is known in Europe as leafspot (*1*) [3] and in Australia as ringspot (*19*). The term "ringspot" has been accepted by different writers in America (*15*, *20*), and since it is fairly descriptive of the lesions it will be used by the writer.

This disease has been known for a long time in Europe, the causal organism having been described by Chevallier (*6*, *p. 449*) in 1826. The first record of its occurrence in the United States is in a paper by Osmun and Anderson published in 1915 (*20*). However, the oldest cauliflower growers in the Colma section state that the disease has been present there for 30 years or longer. It seems probable that it was either brought from Europe by some of the early immigrants or was imported with the seed of some crucifer.

Although this disease is widely distributed in continental Europe and has been reported from England, Wales (*1*), Australia (*19*), New Zealand (*17*), and South Africa (*12*), it does not seem to be very widespread in America. So far as known, it occurs only in the San Francisco Bay region and in Oregon. The disease is very prevalent on the west side of San Francisco Bay, but only an occasional spot is found on the east side. The writer has found the disease as far south as Redwood City, Calif. D. G. Milbrath, of the California State Department of Agriculture, informed the writer that he had found it as far south as Santa Cruz. The disease was prevalent in some fields of cauliflower near Roseburg and Portland, Oreg., during the winter of 1923, while other fields showed little or no infection.

ECONOMIC IMPORTANCE

It is apparent that the premature defoliation produced by the ringspot causes more or less loss to the growers of cauliflower or other cruiferous crops. However, since the disease attacks all of the plants in a field so uniformly, it is practically impossible to determine the reduction of growth due to the disease. In no case has a total loss of a field or even a plant been observed which could be attributed to the disease. Even badly affected cauliflower plants often produce good heads. When the season is favorable for the development of the cauliflower it grows so luxuriantly that the loss of a few of the lower leaves seems to cause little injury. On the other hand, when there is a large amount of rain, the disease develops very abundantly and the growth of the plants is retarded both by the water and by the loss of the lower leaves due to the disease. Even when the spots are not sufficiently numerous to cause defoliation, as is often the case, a certain proportion of the leaf area of a plant is killed, which inevitably results in the production of a smaller head. It seems safe to estimate the losses caused by this disease under varying conditions, due to a reduction in the size of the head, at from 1 to 25 per cent of the crop.

When plants are badly affected, many spotted leaves go into the crates and thence to the markets. These disfigured leaves detract from the appearance of the heads and no doubt render them less salable. It has been observed, however, that shippers seldom object to the spotted leaves as long as the heads are good.

[3] Reference is made by number (italic) to "Literature cited," p. 131.

Walker (*26*) states that ringspot is very common in England; that it is of little consequence on cabbage but that it is sometimes destructive to cauliflower. He also reports ringspot as the most common disease of cabbage in Denmark, where the leaves, stems, and pods often become badly spotted, and in extreme cases the yield may be reduced considerably.

McAlpine (*19*) states that ringspot is very common on cabbage and cauliflower in Australia, but as it occurs only on the older leaves and especially on those which are already fading and withering, it is not regarded as serious; yet he thinks that since the outer and older leaves die prematurely, the plant is bound to suffer from defective nutrition.

Kirk (*17*) regards this disease as being greatly on the increase in New Zealand and the attacks more virulent each year.

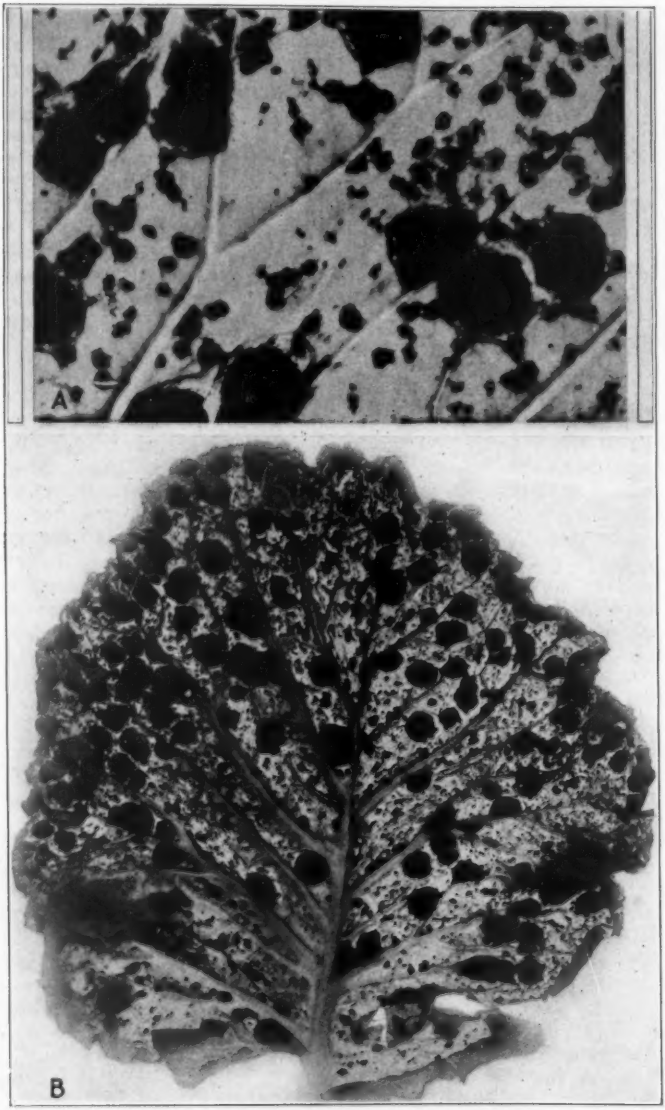
This disease is considered by Osmun and Anderson (*20*) as very destructive to cauliflower in transit.

SYMPTOMS

With the exception of the cotyledons and the young leaves at the center of the plants, practically all parts of the various hosts are susceptible to infection. The laminae of the lower leaves are most often and most severely affected, although the flower stalks, the seed pods, the seeds, the midribs of the larger leaves, and even the sepals are sometimes attacked. The number of lesions per leaf varies from one to several hundred. In extreme cases practically the entire leaf may be involved and killed. The amount of infection varies with the age of the leaves, it becoming less toward the center of the plant. Infection first becomes evident as very small dark-colored spots surrounded by a band of affected tissue having a water-soaked appearance. These have a diameter of one-half millimeter or less when first observed. These spots, as in the case of older ones, are visible from both sides of the leaf. They continue to enlarge, the ultimate diameter varying with the number of lesions per unit of leaf area and other conditions. The average spot bearing mature spores varies from 0.5 to 1 cm. in diameter, although a diameter of about 2 cm. is sometimes reached.

Two types of lesions are often formed (pl. 1, A). The most common one is circular in outline, with a quite definite margin, while the other is more irregular both in shape and outline. During the wet winter of 1923 the latter was the more prevalent, especially on cabbage. Isolations showed these spots to be due to the ringspot fungus. A similar irregularity of the growth of the fungus in culture has been seen (pl. 5, A). A spot is often delimited by a vein. The lesions may coalesce, thus involving and killing considerable areas of the leaf. Likewise, the edges of the leaves are so heavily infected at times that they die and curl upward and inward. The dead tissues become dry, and crack, and pieces break out leaving a ragged margin, as illustrated (for cabbage) in Plate 1, B.

The color of the spots varies with the conditions under which they are developed and the number of fruiting bodies formed. Excluding from consideration the fruiting bodies of the fungus, the color of the lesions varies from sepia to clay (Ridgway's (*22*) color standards). The pycnidia and perithecia are sometimes so abundant as to make the lesions appear almost black. There may be a sharp line of de-



A.—Section of black-broccoli leaf bearing numerous ringspot lesions resulting from natural infection. Both the large circular and the smaller irregular types of lesions described in the text are illustrated. About natural size

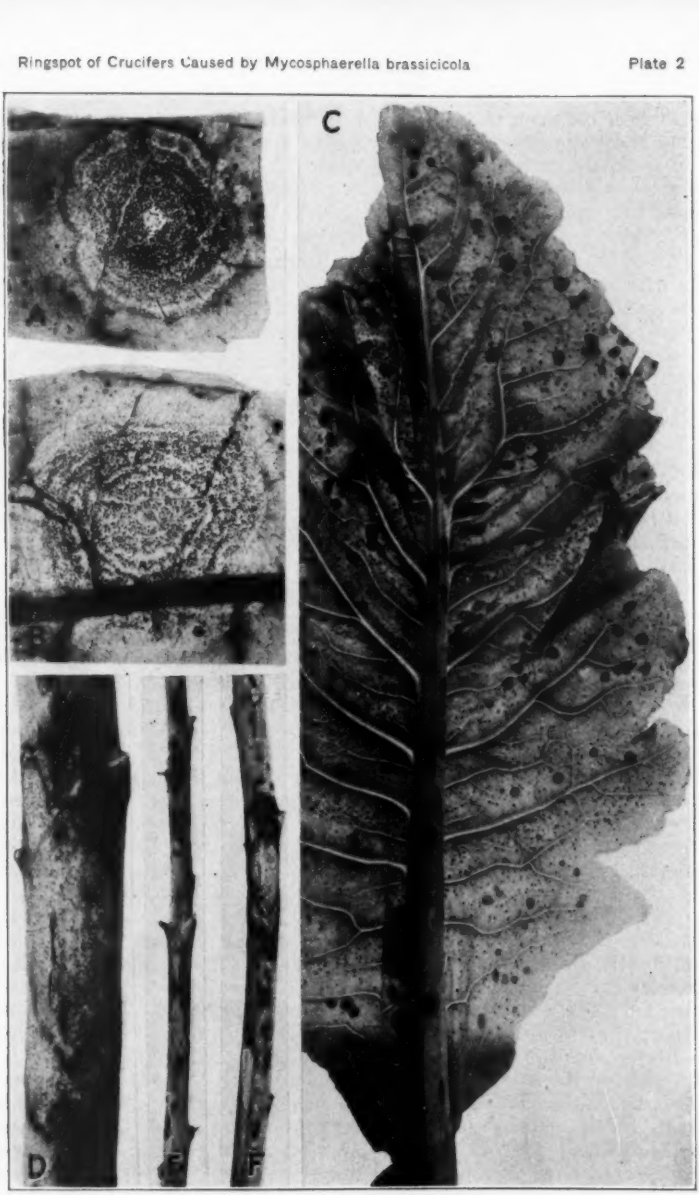
B.—A cabbage leaf very heavily infected along the margin, death of the tissues resulting. These dead areas often roll upward and inward, fragments eventually dropping out, leaving irregularly notched margins. The small, dark spots are young ringspots, or spots which failed to develop. About one-third natural size

marcation between the diseased and healthy tissues. On the other hand, the spots may have an olive-gray border 0.5 to 1 mm. in width. The leaf surrounding the lesions may vary in color from the normal green to yellow ochre to the brown characteristic of the dead leaf. When the spots are sufficiently close the yellow areas about them may coalesce, thus causing a considerable area of the leaf to turn yellow. On the other hand, badly diseased leaves often show little or no yellowing of the noninfected tissues for a long time. Leaves with hundreds of lesions containing pycnidia or perithecia with mature spores often show no appreciable effect of the fungus beyond the spots themselves. The difference is probably due to the variation in the vitality of the leaves.

The mature lesions are thickly studded with pycnidia or perithecia, or both. Either may occur in green or yellowed leaves. The fruiting bodies are present on both surfaces of the lesions, although they are usually more abundant on the upper side. The pycnidia and perithecia may be arranged in concentric rings or scattered promiscuously over the lesion (pl. 2, A and B). However, few if any pycnidia or perithecia are present in the center. Furthermore, small elevations in the form of concentric circles are often present. Three or four bands of color may appear in a single spot, there being a brownish center, then a ring of gray approximately a millimeter wide, and then a band of brown or a different shade of gray. These, together with the concentric arrangement of the fruiting bodies which sometimes occurs, have suggested the name "ringspot."

Not only does this disease attack the laminae of the leaves but the midribs may also be affected (pl. 2, C). These lesions are most abundant on the undersurface of the midribs. These spots are roughly rectangular in shape, the longest sides being parallel to the midrib, and are slate to dusky neutral-gray in color. They are not very sharply delimited from the healthy tissues, neither do they have an entire margin. The spots vary considerably in size, some being just visible macroscopically, while others reach a length of 2 cm. and a width of 0.5 cm. The surface of these lesions may be smooth or slightly depressed. In yellowing leaves, the lesion may be slightly elevated. Lesions similar to those on the midribs occur more or less abundantly on the flower stalks. Although these spots are often irregular in shape, they are frequently elliptical or rectangular. In either case, the longer axis runs parallel with the stem. As the lesions develop they become brownish to gray in the center and more or less thickly studded with pycnidia or perithecia (pl. 2, D, E, and F). The spots on the stems sometimes reach a length of 2.5 cm. and extend entirely around the stem. Several lesions varying in size may be present on the pods, as shown on cabbage pods in Plate 3, B. These sometimes coalesce and involve large areas or even the entire pod. The affected parts of young pods may become shrunken and the pod may die. Often the disease extends entirely through the side of the pod, discoloring it (pl. 4, A) and affecting the seeds, which may be slightly discolored and shriveled. As the pod matures and dies the fungus often invades all parts of it, and it becomes covered with pycnidia or perithecia (pl. 3, C).

Occasionally sepals are affected with ringspot. The affected parts are only slightly discolored and may be studded with pycnidia. No infections were found on any of the other floral parts.

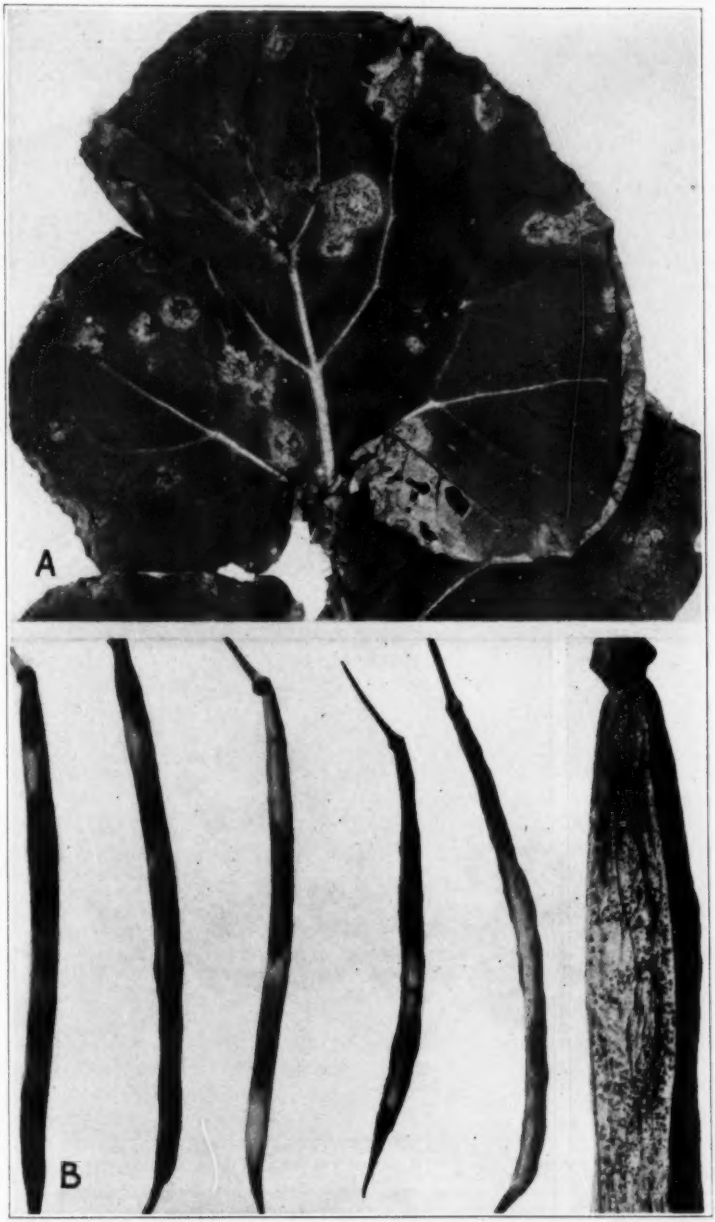


A and B.—Two lesions on a cauliflower leaf decolorized with 95 per cent alcohol and 5 per cent NaOH, to show the distribution of the fruiting bodies, which are scattered more or less uniformly over the surface in A and are arranged in concentric circles in B. Note in B that the vein failed to intercept the spread of the fungus, as is frequently the case. The small dark spots are young lesions. × 2½

C.—Underside of a black-broccoli leaf, showing the type of lesions produced on the midrib by *Mycosphaerella brassicicola*. Also, ringspot lesions in different stages of development in the laminae of the leaf. × ½

D.—An enlargement of the large grayish lesions shown in F. The pycnidia are scattered promiscuously over the surface. × 2½

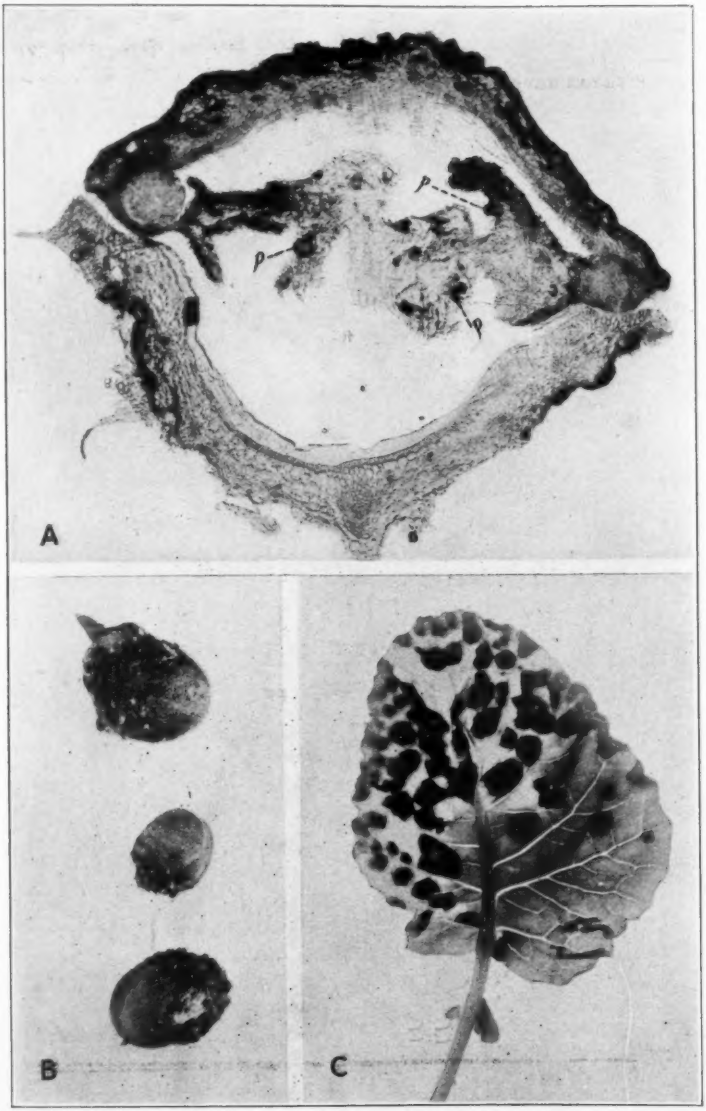
E and F.—Photograph of portions of cabbage flower stalks with characteristic ringspot lesions. Note the large grayish lesions in F. × ¾



A.—Leaf of yellow turnip affected with ringspot. The lesions are paler in color and usually bear fewer fruiting bodies here than on other hosts. About natural size

B.—Cabbage seed pods, showing dark-colored ringspot lesions of varying sizes. × 1

C.—Portions of old seed pod enlarged. The fungus has permeated the entire pod, and perithecia and pycnidia are scattered promiscuously over the surface. × 5



A.—Free-hand cross section of an infected cabbage seed pod. All the darkened parts are diseased. Pycnidia were present inside the pod, as indicated by letter P. × 30

B.—*Mycosphaerella brassicola* growing from cabbage seeds removed aseptically from diseased pods and planted on agar. × 6½

C.—A young black-broccoli leaf from a plant brought from the field and planted in the greenhouse on Jan. 9, when the spots were mere points just visible to the unaided eye. Photograph shows the spots as they appeared Jan. 30. The light part of the leaf was yellow, and the rest normal green. × ⅔

THE CAUSAL FUNGUS

NAME

The fungus causing this disease was first described by Chevallier (*6, p. 449*) in 1826 as *Asteroma brassicae*. In 1830 Duby (*4, p. 712*), attributing the name to Fries (ined. in litt. ad Cl. Moug.), published a description of the same fungus as *Sphaeria brassicaecola*. He does not state whether or not Fries wrote the description. Berkeley and Broome in 1852 (*2, p. 384*) listed the fungus *Sphaeria brassicae* as equivalent to *Asteroma brassicae* Chev. In 1860 Berkeley (*3, p. 401*) mentioned this fungus under the name *Sphaeria brassicaecola*, having this time accepted the specific name first applied by Fries. Cesati and de Notaris (*5*), in 1863, changed the generic name of this fungus to Sphaerella, retaining the specific name of *brassicaecola* and listing *Sphaeria brassicaecola* Fries Ap. Duby and *Dothidea brassicae* Desmaz. (*11*) as synonyms. Saccardo (*23, p. 502*) called this fungus *Sphaerella brassicicola* (Duby) Ces. et de Not., but gave as a synonym *Sphaeria brassicaecola* Fr. et Duby, thus giving the credit for the name in one place to Fries and Duby and in the other to Duby alone. On the other hand, Lindau (*18, p. 423–426*) in 1897 explained that the name Sphaerella could not be used, since it had been previously applied to a genus of the Volvacaceae, and adopted the name Mycosphaerella, which had previously been proposed by Johanson. This makes the correct name of this fungus, according to Lindau, *Mycosphaerella brassicicola* (Duby) Lindau. There seems to be some difference of opinion regarding the author of the specific name, but since Duby himself gave the credit to Fries, the writer is inclined to do the same and write the name *Mycosphaerella brassicicola* (Fr.) Lindau.

The imperfect stage of this fungus was first described as an Asteroma by Chevallier (*6, p. 449*), but Grove (*14*) states that it is not an Asteroma, because it has no "fibres" surrounding the pycnidia, which fact, according to Grove, was recognized by Berkeley in 1841. Grove also states that "it seems at times to come rather under the head of Gloeosporium, since the pycnidial envelope is then very delicate and somewhat imperfect." He describes it, however, as *Phyllosticta brassicicola*, the name which had been previously applied to it by McAlpine (*19*).

PATHOGENICITY

Although *Mycosphaerella brassicicola* is commonly accepted as the fungus causing ringspot, no proof of its parasitism has been found in the literature. Work done on this disease seems to be limited largely to field observations, where it is evident that this fungus is constantly associated with the typical ringspot lesions. In order to prove beyond doubt that the fungus associated with the diseased spots is the causal organism, the following studies were made:

Method of Isolation

Portions of tissue were cut from the leaves, which had been washed previously with soap and tap water. These were immersed in a solution of equal parts of 95 per cent alcohol and 1–1,000 $HgCl_2$ for one-half to two minutes. They were then washed thoroughly in sterile distilled water, cut into small pieces, and placed on agar in

Petri dishes. After a week or 10 days, a transfer of the mycelium was made to agar slants. Secondary fungi, usually a species of Macrosporium, with a Pleospora stage developing later, were often obtained from the older lesions.

Another method of isolating the causal organism was as follows: Portions of heavily infected leaf containing mature perithecia were washed with soap and tap water and then suspended in moist chambers over Petri dishes containing agar. In 12 to 18 hours numerous spores were present on the surface of the agar in the Perti dishes. Directly beneath some lesions the agar was almost completely covered with spores, the number gradually diminishing away from these centers. Selecting a place where there were few spores, single spores were removed to agar slants in test tubes.

Inoculation Experiments

Numerous attempts were made during the winters of 1922–23 and 1923–24 to infect cauliflower and cabbage plants growing in the greenhouse and in the garden on the campus of the University of California. Small pieces of mycelium from pure culture were placed on the leaves of the plants, which were then covered with battery jars and kept moist for three or four days. Some leaves were injured and the mycelium placed in wounds. In other instances leaves containing numerous perithecia were suspended over moist plants beneath a bell jar. Although numerous inoculations were made during the season of 1922–23, no infection was obtained where mycelium was used and only a few spots developed in two of the trials where the leaves were suspended over the plants. On December 8, 1923, a heavily infected cauliflower leaf was suspended over a young cauliflower plant which had been atomized with tap water and the plant was then covered with a bell jar and thus kept wet for three days. On February 5, 1924, two nearly mature ringspot lesions were found on two of the lower leaves of this plant. No doubt the spots had been evident for some time but had been overlooked. It should be stated that these inoculation tests were all made on plants growing on soil which had not grown cauliflower for years, if ever; hence the soil can safely be said to have been free of the ring-spot fungus. No diseased cauliflower plants were known to be growing within a radius of 15 or 20 miles.

On January 14, 1924, two plants about a foot high were inoculated with bits of hyphae from a pure line culture. Slight injuries were then made in the surfaces of the leaves, and small pieces of hyphae were placed therein. These were then covered with strips of wet filter paper over which small pieces of wet cotton were placed. The plants were then kept covered with large battery jars for five days. No infection was noticed on February 11, but on February 16 one large and two small lesions with pycnidia already evident were found developing in one of the inoculated spots on one of the leaves. Isolations were made and the fungus was recovered and identified. In this experiment thirty-three days elapsed between the time the inoculations were made and the time the infection was first observed. On March 1 another lesion was found, this time on a leaf of the other plant inoculated on January 14. This spot was somewhat elliptical

in shape, 8 by 10 mm. in diameter, and contained numerous pycnidia. Infection was obtained subsequently in this manner several times, although numerous attempts failed.

A plant which was inoculated on February 4, 1924, by suspending an infected leaf over it and then covering it with a bell jar for several days, showed numerous typical ringspot lesions on March 10. On February 5 four plants were sprayed with a suspension of ascospores in tap water. Two of these plants were covered with battery jars, while the other two were left uncovered. The uncovered plants remained wet almost continually for three days and three nights, due to fogs and rains. On March 8 several ringspot lesions were present on the lower leaves of both of the plants left uncovered, but none were present on the leaves of the two plants which had been covered. Evidently the conditions of the outside air were more favorable for infection than those beneath the battery jars, although droplets of moisture were present on the leaves of the covered plants throughout the entire period.

On February 5 cauliflower leaves with numerous ringspot lesions containing perithecia were laid upon 12 cauliflower plants, which were 12 to 18 inches high. The fog and rain kept the plants wet for three days and nights, thus giving ample opportunity for spore discharge and infection. The first evidence of infection was noticed on March 1 in the form of numerous small spots from 1 to 4 mm. in diameter. A week later the lesions varied from 1 to 7 mm. in diameter, and fruiting bodies were evident. This experiment shows that about three weeks may be expected to elapse after inoculation before definite lesions large enough to be recognized as those of ringspot become evident, and that fruiting bodies do not become visible for nearly a month.

Likewise, similarly infected leaves were placed upon cabbage (Columbo variety) seedlings in a seed bed. Very young spots about 0.5 mm. in diameter were noticed at the end of a week. However, not until nearly three weeks had elapsed did these spots take on the characteristic appearance by which ringspot lesions may be distinguished from those caused by species of Alternaria. On February 6 four large cauliflower plants were sprayed with an atomizer with a suspension of ascospores in tap water. The plants were left uncovered, but were kept wet by fog and rain for two days and nights. No infection was evident on March 1, but a few spots were found on the lower leaves of two of the plants on March 8.

An attempt was made on February 16, 1924, to infect 4 cauliflower plants about 4 inches high by placing hyphae from pure agar cultures on injured places in the leaves. Ten inoculations were made on each plant, making 40 in all. The plants were covered with bell jars for three days and nights. The plants were atomized with sterile distilled water at the time of inoculation and again at the end of the second day. Only one characteristic ringspot lesion developed.

Numerous other inoculation experiments were conducted with both cauliflower and cabbage. However, the experiments just given in detail are sufficient to show the methods used and something of the rate at which the infection develops. Only under the most favorable weather conditions (fairly low temperature and very high humidity) could infection be obtained.

One other method by which infection was obtained might be mentioned here. This consisted in placing cabbage seed pods infected with ringspot and containing numerous perithecia beneath cauliflower plants growing in a seed bed. Numerous typical ringspot lesions developed on the lower leaves of several of the plants.

LIFE HISTORY

Spore Forms

Both the pycnidial and perithecial stages of this fungus may be found in abundance on green and vigorous leaves in the Colma section. This does not seem to be true in all countries. Grove (*14*) states that the perfect stage is rarely found in England and then chiefly on the old or dead leaves. Cooke (*9, p. 82–83*) also mentions the fact that the perfect stage is not reached until the leaves have lain on the ground. Berkeley and Broome (*2, p. 384*) record the infrequent occurrence of the perfect stage of this fungus. On the other hand, Kirk (*17*) finds the ascospore stage to be quite common in New Zealand and thinks that this may account for the fact that the disease is proving more troublesome there than in Europe, where the perfect stage is rare. McAlpine (*19*) states that in Australia pycnidia are most common in autumn and winter. This stage appears on still green, fully grown leaves, and is the only one present in late autumn. However, he finds the perfect stage developing on dead and fallen leaves at the beginning of winter. Not only are both the pycnidial and perithecial stages present on green, vigorous leaves in America, but both stages are present during the entire year. It is true, however, that the perithecial stage is most abundant during the autumn, winter, and spring months, from November to May, while the pycnidial stage is most prevalent during the remainder of the year. This varies somewhat from year to year, depending, no doubt, on weather conditions. High humidity and low temperature seem to be conducive to perithecial development.

Spore Discharge

Studies were made to determine whether the ascospores were forcibly ejected from the perithecium, and, if so, to learn something regarding the conditions under which this takes place. In all of these experiments the method of procedure was as follows: Pieces of lesions containing perithecia were floated upon tap water placed in a cell made by sealing a glass ring about 1.5 cm. in diameter and 1 cm. high to a microscopic slide. A cover slip was then sealed onto the ring with vaseline. The distance between the leaf tissue and the under side of the cover slip was varied by removing or adding water to the cell. Sufficient moisture condensed on the cover slip to hold many of the discharged spores and permit their germination. Most of these tests were conducted at room temperature (18° to 22° C.). The results of several experiments of this nature showed that the ascospores are forcibly ejected in large numbers from a very small portion of a lesion. Only one spore is discharged at a time, followed a short time later by another and then another until all eight are discharged. Perithecia contain spores in all stages of development,

so a single perithecium continues to discharge spores intermittently over a considerable period. On one occasion a lesion 4 by 8 mm. in dimensions was mounted on water, and the arrival of the spores on the cover slip above was observed under the microscope. Approximately 100 spores were caught in 10 minutes. This cover slip was replaced by another on which literally hundreds of spores were caught in the course of the next hour and a half. A large number of spores were caught when the leaf tissue was 4 mm. below the cover slip; some were caught at a height of 5 mm.; but few were caught at a greater height.

A slight modification of the above method was used to study the influence of temperature on spore discharge. In this case the lesion was placed on a single large drop of water in the center of the ring at the bottom of the cell. Four cells, each prepared in this manner, were placed in an inverted position at different temperatures. Since the cells were inverted all of the spores discharged fell on the cover slips. Likewise the danger of washing the spores from the cover slips by jarring, while moving the cells about, was obviated. The following temperatures were used: 0°, 2.2°, 4.5°, 7°, 12 to 14°, 15 to 20°, 24°, and 27° C. An examination after 24 hours showed that some spores had been discharged at all of the temperatures except 0°, 2.2°, 4.5°, and 27.5°. However, after 48 hours some spores were present on some of the cover slips at all of the temperatures except 27.5°. At 0° spores were found on but one cover slip, while at 2.2° they were present on all four. However, as many spores had been discharged in the one case at 0° as at 2.2°. No reason for the failure of spores to be discharged in three out of four cases at 0° was apparent, although failures of this nature occurred in some tests due to insufficient moisture, chiefly at the higher temperatures, or to the fact that the spores were not mature and hence not ready to be discharged. This experiment shows that spore discharge will take place at temperatures ranging from 0° to about 25° C.

An attempt was made to determine whether the ascospores could be caught in the field, and, if so, to learn something of the conditions under which they were disseminated. Petri dishes coated with glycerine were placed on the ground between the rows of badly diseased cauliflower plants. The plants were large, the overhanging leaves filling practically the entire space between the rows. The plates were placed in position at 8 a. m. and were left exposed for 2 hours, after which they were replaced by others, which were in turn supplanted by others two hours later. Thus plates were exposed from 8 to 10, from 10 to 12, and from 12 to 12.45 p. m. At the end of the exposure they were examined microscopically for the presence of spores. Ascospores were found in considerable abundance in all of the plates. These were most numerous in the plates exposed from 8 to 10, only slightly less numerous in those exposed from 10 to 12, while comparatively few were present in those exposed from 12 to 12.45. There had been no rain for four days prior to the day on which this test was made, but the cauliflower leaves were wet with dew. They remained wet until about 10 o'clock, when the upper ones began to dry off, so that by 12 only the lower leaves remained wet. No record of the temperature was obtained, but it was judged to be between 45° and 50° F. This experiment shows that ascospores were being dis-

seminated in large numbers throughout the forenoon. It seems probable that ascospores were being discharged in large numbers during this time. However, it is possible that many of the spores had been lying on the leaves and were dislodged by the wind.

Two subsequent tests were made, using a larger number of Petri dishes each time. These dishes were placed over and under infected leaves, at various heights from the ground and in many positions relative to the infected plants and fields. Conditions during these tests seemed to be favorable for spore dispersal, but in no case were any spores found on the plates. These experiments indicate that spore dispersal is not taking place continually, but that when the conditions are just right they are discharged and disseminated in large numbers. They also show that rain is not a necessary preliminary to this phenomenon.

Regarding the dispersal of the pycnospores, Osmun and Anderson (*20*) state that "the pycnospores are formed in enormous numbers and ooze out through the ostiole in a pink, gelatinous thread." Kirk (*17*) says the pycnidia contain immense numbers of minute spores which are ejected from the tops of the pycnidia in small more or less mucilaginous tendrils. This method of pycnospore discharge has never been observed by the writer, although careful watch had been kept for it.

Leaves containing pycnidia were often brought into the laboratory, washed, and then placed in moist chambers and kept under observation for several days. Likewise, on numerous occasions, lesions containing pycnidia were sectioned and examined microscopically in water. In no case were pycnospores found oozing from the pycnidia in tendrils. Often a dozen or more spores would be seen in the water at the ostiole, but they never appeared in great numbers. Although a total of hundreds of pycnidia have been sectioned at different times, only now and then was one found which contained many spores. Owing to the scarcity of this type of spores, none were ever obtained for careful study. When mounts of sectioned pycnidia showed a few pycnospores these were kept under observation. In no case did any germination occur, even after a period of three or four days.

Dissemination

by mechanical means

There seems little doubt that the wind is the chief agent in disseminating the causal organism. Many of the cauliflower leaves stand nearly erect, so that ascospores ejected only a short distance can easily be caught by the wind and carried away. Any spores lying on the leaf could be readily jarred loose. The cabbage and cauliflower fields in the Colma section are so near to one another that dissemination over long distances is not necessary. The leaves of these plants are wet with dew or fog a large proportion of the mornings of the year. Often drops of water which may contain either asco or pycnospores roll down a leaf and fall upon those below or are blown by the wind to neighboring plants. The workmen when walking through the fields while the plants are wet, for the purpose of cultivating, irrigating, or harvesting the crop carry the spores about.

ON SEEDLINGS

By the time the plants are ready for transplanting, numerous spots, often containing perithecia, are frequently present on three or four of the lower leaves. Usually some of these lower leaves fall off soon after transplanting. However, the fungus may be present in some of the leaves which remain attached to the plant for a considerable period; in any case the fungus in mature form in the leaves beneath the plants serves as a source of infection. The disease may be transported long distances in this manner.

IN THE SEED

As has been stated, the seed pods may be attacked and the fungus may grow through the seed pod into the seed (pl. 4, A and B). After thoroughly washing and disinfecting the pods in $HgCl_2$ 1 to 1,000, the fungus was isolated from seed taken from cabbage-seed pods which had not yet begun to dehisce. The infected seeds were transferred from the agar plates to slants in test tubes, where in a few instances the seeds grew. Only in one or two cases did the fungus appear to have grown through the seed coat into the cotyledons. However, the pods and seeds used in these experiments were still green. It is possible that if older seeds had been used the fungus would have been found more frequently in the cotyledons. Nevertheless, since careful search on numerous occasions has failed to reveal a single instance of cotyledon infection in the seed bed, the writer believes that seed infection by this fungus is commonly limited to the seed coat.

IN THE SEED PODS

An experiment was conducted to determine whether pieces of seed pods found with the seed after threshing could serve as a source of infection. On November 13, 1923, a quantity of the infected pods which had been in the laboratory for five weeks was crushed into small pieces and scattered beneath healthy young cauliflower plants in a seed bed. Due to the dry weather, no infection appeared until February 11, 1924, at which time one leaf had six typical ringspot lesions. Mature ascospores developed in these a short time later. On February 13 a number of other lesions were found on other plants and the disease continued to spread, and by March 10 a large number of infections were present. No doubt some of those which developed later in the season were secondary, resulting from the ascospores formed in the primary lesions. This experiment repeated later in the season showed that the fungus in the seed pods was still capable of causing infection four months after the seed was harvested. These experiments show how important it is that the seed be freed from all débris before planting. They also indicate that the causal organism may be carried with the seed to other parts of the country or to foreign countries. No doubt this method of distribution is responsible for the appearance of this disease in isloated localities in Oregon.

IN THE LEAVES

Experiments mentioned under the heading "Pathogenicity" proved that the disease could be transmitted to healthy plants by laying diseased leaves over them. An experiment was conducted to determine whether infection would result from placing diseased leaves beneath cauliflower plants in the same manner as the seed pods were placed.

This experiment was carried out simultaneously with the seed-pod test, and with similar results. However, not such a heavy infection developed as where the seed pods were used. The leaves disintegrated more rapidly than did the seed pods, which makes the former less likely to harbor the causal fungus over a long period of time under field conditions. Nevertheless, the fact that some disease developed in this experiment shows that diseased leaves are also a source of the disease as far as being able to carry the inoculum is concerned.

IN THE SOIL

Several attempts were made to determine whether the disease could be distributed or carried over from one crop to another by means of the soil. Soil from fields on which a heavily infected crop of cauliflower was growing was placed in pots, some of which were held in the greenhouse and others out of doors. Cauliflower seed was sown in these pots, and close watch was kept for any signs of disease. In no case did any disease develop under the conditions of these experiments.

INFECTION

METHOD OF PENETRATION

In order to determine the method of penetration by the germ tube, the following experiment was performed: A glass ring was placed upon a leaf of cauliflower held in a moist chamber. A piece of a ringspot lesion containing many perithecia was placed on a drop of water in the center of a cover slip, which was then sealed on the glass ring with vaseline. This suspended the lesion over the cauliflower leaf in such a way that the spores discharged were caught on the leaf. Likewise, the ring helped to keep the cauliflower leaf moist enough for the germination of the discharged spores. After 72 hours a microscopical study was made of the portion of the leaf beneath the lesion, to see whether any spores were present, and, if so, whether they had germinated and entered the leaf. A number of spores were found, most of which had germinated. Only one of the germ tubes could be definitely traced as it entered the stomatal pore. A camera-lucida sketch of this is seen in Figure 1. No evidence of direct penetration could be found. It is believed that the fungus normally enters the host through the stomatal pore.

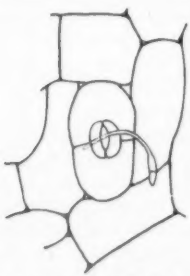


FIG. 1.—Camera-lucida sketch, showing the germ tube of an ascospore of *Mycosphaerella brassicicola* entering a stoma of a cauliflower leaf. × 250

STOMATAL STUDIES

It has been commonly observed that the innermost leaves of all of the hosts are not susceptible to ringspot. As stated above, the cotyledons seem to be very resistant, if not immune, to infection. Likewise, the first pair of true leaves are resistant for a time but soon become susceptible. Small plants may have leaves with laminae 2 inches long which are susceptible, while large plants may have leaves with laminae a foot long which are resistant. Size is no measure of the resistance of the leaves.

Portions of the epidermis were stripped from leaves of cauliflower, plunged at once into absolute alcohol, and then the number of stomata as well as the size of the pores determined. In this way the stomata from leaves of different ages, both susceptible and immune, were studied. It was difficult to determine just when a leaf became susceptible, as the fungus developed rather slowly. Therefore some leaves were selected for study which showed the very earliest signs of disease, namely, very minute spots just visible to the unaided eye. For purposes of comparison, other leaves which showed no evidence of infection were taken from slightly nearer the center of the plant. Likewise, the stomata from old leaves grown in the field and in the greenhouse, as well as those from cotyledons, were studied. The data thus obtained show that the infected leaves have in every case from 142 to 300 stomata per square millimeter of leaf surface, while the noninfected leaves have 342 to 575. These data also show that the pores in the susceptible and immune leaves were about the same size, namely, from 3.5 to 7.5 μ in width. In some cases the stomata in very immature leaves were found to be closed and undifferentiated. However, the older noninfected leaves, as well as the older cotyledons, had fully mature stomata with open pores as large as or larger than those in the older leaves. Since the germ tubes are from 1.5 to 2.5 μ in diameter, the stomatal-pore size can not be a factor in the resistance of certain leaves. The only consistent point of difference between the noninfected and infected leaves was the number of stomata per square millimeter of leaf surface. The leaves having 300 or fewer stomata per square millimeter were susceptible, while those with more than this number were resistant. This probably means that by the time the leaves reach a stage of maturity at which they have approximately 300 stomata per square millimeter they have undergone some physiological change which renders them capable of being attacked by the fungus. A similar correlation between the number of stomata per square millimeter of leaf surface and infection of sugar-beet leaves to *Cercospora beta* was found by Pool and McKay (*21*).

EFFECT OF TEMPERATURE ON ASCOSPORE GERMINATION

The method employed was for the most part the same as that used for the study of spore discharge, which consisted in placing the lesion bearing the perithecia on a drop of water on the slide in the center of a ring sealed on a microscopic slide. The slide was inverted so that the spores were caught on the cover slip. In one experiment at room temperature (20° to 22° C.) spores were ejected and germ tubes 25 μ long had formed in 18 hours after the experiment was set up. These germ tubes had increased in length to 70 μ in 30 hours. This indicates

that spores can germinate at this temperature and germ tubes sufficiently long to cause infection be produced in 18 to 30 hours or less, depending on the distance the spores lie from the stomata. The results of similar tests made at various temperatures are given in Table I.

TABLE I.—*Effect of temperature on ascospore germination*

Temperature (°C.)	Time of exposure, in hours [a]	Percentage of germination	Length of germ tube
28	24	0	
28	48	0, spores dead	
24	24	1	Just starting.
18 to 22	24	10	Longest, half as long as spore.
18 to 22	48	75	From just starting to twice the length of spore.
7 and below	24	0	
7	48	10	From just starting to twice as long as the spore.
7	96	50 to 75	Longest, 5 to 6 times as long as the spore; average about 2.5 times as long as spore.
4.5	48	5	Longest, half as long as spore.
2.2	48	1	Just starting.
0	96	0	

[a] This includes the time for spore discharge as well as for germination.

Few temperatures were available, but such as were tried indicate that the optimum lies somewhere between 15° and 22° C. There was a rapid decline in the rate of germination at 24° and the spores are killed at 28°. Good germination took place at 7°, but this process was much retarded at this temperature. Some germination took place at temperatures as low as 2.2°, and possibly would have occurred at still lower temperatures if sufficient time had been allowed. This means that infection can take place at quite low temperatures, providing the plants are kept wet over a sufficiently extended period.

EFFECT OF TEMPERATURE ON MYCELIAL GROWTH

A study of the effect of temperature on the growth of *Mycosphaerella brassicicola* in pure culture was made also. In order to have approximately equal quantities of inoculum for each inoculation and to have the inoculum as nearly uniform as possible, a small piece of mycelial felt was crushed in a test tube of melted agar, which was then poured into a sterile Petri dish. After five days, at which time these segments of mycelia showed a slight growth, pieces of uniform size were transferred to 100 c. c. flasks containing 1 per cent dextrose agar (2 per cent), which were then held at different temperatures. Two flasks were held at each temperature.

The data in Table II show that temperatures varying from 15 to 22° C. were the most favorable of those tried, although fair growth took place at 24° and at 12° to 13° C. These data also show that growth is very slow at 7° and extremely slow at 4.5° or below. Nevertheless some growth was made even at 0° in 45 days, and a sclerotiumlike body 11 by 5 mm. in dimensions was formed in one year. Transfers from this year-old culture grew, showing that the fungus can remain alive and make some growth at 0°. Not only was the fungus able to live and grow at 0°, but it also formed pycnidia with a few pycnospores of normal size. The fungus was killed at 30° in 15 days or less, as indicated by the fact that it failed to grow when

removed to a temperature favorable for its growth. It should be stated also that the diameter of the colony formed is not necessarily a true measure of the growth of this fungus, as it tends to form a thick sclerotiumlike felt rather than to spread over the surface of the medium as do many fungi.

TABLE II.—*The effect of different temperatures on mycelial growth after 15 and 45 days*

Temperature (° C.)	Observations after 15 days	Observations after 45 days
30	No growth; fungus dead, as shown by its failure to grow when placed at a favorable temperature.	
24	Diameter of culture 6 to 7 mm	Growth continued, but agar too dry for good growth.
15 to 22	Diameter of culture 8 mm	Culture 15 by 18 mm. in diameter.
12 to 13	Diameter of culture 6 to 7 mm	Culture 12 by 15 mm. in diameter.
7	Growth very slight	Diameter of colony 2 to 3 mm.
4.5	Growth very slight, if any	Slight growth evident.
2.2	No visible growth	Do.
0	do	Do.

As stated previously in this paper, the distribution of this fungus in America is limited to the cool Pacific coast sections. Although it is present on the eastern side of San Francisco Bay, it appears there to only a very limited extent. It is thought that either temperature or humidity or both may be factors in preventing the development of the fungus in this section. In order to learn more of the limiting effect of temperature an attempt was made to determine more definitely just what temperature the fungus can endure. Previous experiments had shown that the spores were killed at 28°C. and that the mycelium was dead at the end of 15 days at 30°. Hence an attempt was made to see how long the fungus would live at 32° to 33°. Sixteen tubes containing beef agar plus 2 per cent dextrose were used. These were placed in water at 32° to bring the agar up to this temperature. After the agar had reached 32°, as determined by a thermometer inserted into an uninoculated tube of agar, one tube was removed at a time, inoculated with mycelium from the same agar slant, and returned to the water bath. After all of the tubes were inoculated they were held in a moist chamber in an incubator at 32° to 33°. Two tubes were removed from the incubator each day after the first and held at room temperature. In the tubes exposed 1 and 2 days, growth was evident 10 days after the experiment was started, while in 5 days growth was observed in control tubes inoculated at the same time and held continuously at room temperature. Growth started subsequently in the tubes exposed for 3 days, thus confirming previous results, namely that the fungus can withstand a 3-day exposure to this temperature. No growth was evident in any of the other tubes after 3 weeks. Hence it may be concluded that this fungus will be killed by a 4-day exposure at 32° to 33°.

Another experiment was performed to determine the effect of alternating versus constant temperatures on the fungus. A number of beef-agar slant cultures of this fungus were made using as inocula small amounts of mycelium obtained as in the previous experiment. Two groups of 30 of these cultures were placed in incubators at the following

temperatures: 31.8° C. (limits 31.2° to 32°), 29.4° (limits 28.6° to 30°), and 26.9° (limits 26° to 28°). One group of tubes was held at these temperatures continuously, while the other was removed at 4 p. m. and held until 9 a. m. at a temperature of 8.8° (limits 7° to 10°). Similarly prepared control cultures also were held at 8.8°. Two tubes were removed practically every day from each group at each temperature and placed at 8.8°. At the end of the experiment all of the cultures were held for 10 days at room temperature (20° to 25°) which permitted more rapid growth. The results may be summarized briefly as follows: The fungus, when held continuously at 31.8° and 29.4°, was dead at the end of 9 days. At 26.9° it was still alive at the end of 24 days, but its growth was very much retarded as contrasted with that of those cultures removed several days earlier. The fungus, when held at 31.8°, 29.4°, and 26.9° for 7 hours a day (9 a. m. to 4 p. m.) and at 8.8° the remainder of the time, was still alive at the end of the experiment and grew normally when removed to a more favorable temperature. Cultures held continuously at 8.8° made a good growth. It is evident, then, that the fungus will be killed in 9 days under the conditions of this experiment when kept continuously at a temperature of 29.4° (28.6° to 30°), but will survive a temperature of 31.8° (31.2° to 32°) for 24 days when exposed to this temperature for only 7 hours a day, a period corresponding to the warmer part of the day.

A temperature of 55° to 56° C. is frequently recommended for treating cabbage and cauliflower seed to kill disease-producing organisms, so it was thought desirable to determine whether *M. brassicicola* in pure culture would be killed at that temperature. Small pieces of mycelial felt were transferred to beef-agar slants, and all but two, which were held as controls, were placed in a water bath at 55° to 56°. A thermometer inserted in a tube of agar showed that 4 to 5 minutes time was necessary to bring the agar on which the fungus lay up to a temperature of 55° to 56°. Therefore an allowance of 5 minutes was made in the time of exposure in each case. Two tubes each were removed from the water bath after 15, 30, 50, and 60 minutes. No growth was made in any but the control tubes after 3 weeks. It may be concluded, therefore, that this fungus, under the conditions of this experiment, will be killed in 15 minutes or less when exposed to a temperature of 55° to 56°.

In order to test the effect of a temperature of 55° to 56° C. on the fungus for shorter periods of time, and also to determine how long the fungus could withstand a temperature of 50°, the following experiment was performed. Small thin-walled test tubes containing 1 c. c. of sterile distilled water were inoculated with a small amount of mycelium and then immersed in water held at a temperature of 55.5° to 56° for 5, 10, and 15 minutes and at 50° to 50.5° for 10, 20, and 30 minutes. The time necessary for the water in the tubes to reach these temperatures was determined and due allowance made. Five tubes were removed at the end of each time interval and plunged at once into cold water. The mycelium was transferred from the tubes of water to agar slants and incubated at room temperature (20° to 25°) for 3 weeks. Transfers of the same inoculum were made directly to agar slants and held with the other tubes as controls. At the end of 3 weeks the controls had made a good growth, but there was no evidence

of any growth in the cultures made with the treated mycelium. Therefore the ringspot fungus can be killed by an exposure of 5 or 10 minutes at 55.5° to 56° or 50° to 50.5°, respectively, under the conditions of this experiment.

The foregoing experiments show that this fungus in pure culture is quite sensitive to heat. Whether it resists high temperatures when within the leaves or other parts of the host or in the soil has not been determined.

RELATION TO WEATHER

Regardless of the fact that it is impossible to analyze all of the factors which may be involved in the development of ringspot, certain weather data are of interest, both from the standpoint of infection and of distribution. Figures given in Table III show that the mean temperatures for San Francisco during the hottest months—July, August, September, and October—are 59.3°, 58°, 59.3°, and 58.4° F., respectively, while the mean maxima for these same months are 63.7°, 63.9°, 67.2°, and 66.8°, respectively. The temperatures on the San Francisco side of the bay are favorable for the growth of the ringspot fungus even during the hottest months. For comparison, the temperatures for San Jose, which are more nearly representative of those of the cauliflower-growing sections on the east side of the San Francisco Bay than any others available, as well as those for Los Angeles, where the disease is not known to exist, are given in Table III. The mean as well as the mean maximum temperatures for both San Jose and Los Angeles are considerably higher during the summer months than these temperatures in San Francisco. Temperatures ranging from 79.9° to 82.3°, as do the mean maxima for San Jose and Los Angeles during July and August, are very unfavorable for this fungus. However, it must be kept in mind that the temperatures drop considerably at night in all three of these places, the mean minimum in all cases being between 50 and 60°.

TABLE III.—*Mean and mean maximum temperatures, and mean relative humidity, for San Francisco, San Jose, and Los Angeles for each month for a varying number of years*

Locality	Mean temperature (°F.)			Mean maximum temperature (°F.)			Mean relative humidity (per cent)		
	San Francisco	San Jose	Los Angeles	San Francisco	San Jose	Los Angeles	San Francisco	San Jose	Los Angeles
Length of record in years	40.0	37.0	42.0	40.0	10.0	42.0	19.0	5.0	32.0
January	49.5	48.3	54.5	54.6	57.9	64.3	81.0	74.0	65.5
February	51.3	50.6	55.5	57.0	61.1	65.2	79.0	67.0	69.0
March	52.7	53.7	57.3	58.8	63.8	67.0	77.5	64.0	70.0
April	53.7	56.7	59.7	61.4	69.9	69.7	77.0	58.0	75.0
May	55.5	60.7	62.1	62.0	72.3	71.6	79.5	55.0	76.0
June	57.0	65.0	65.2	64.0	76.9	76.7	80.5	53.0	75.0
July	59.3	66.9	70.2	63.7	80.9	81.4	84.5	51.0	75.0
August	58.0	66.7	71.1	63.9	79.9	82.3	86.0	53.0	75.0
September	59.3	64.7	69.4	67.2	79.3	80.9	80.5	52.0	72.0
October	58.4	60.3	65.1	66.8	74.5	76.3	78.5	52.0	70.5
November	55.5	54.4	60.9	61.9	65.4	72.3	78.0	61.0	63.0
December	50.9	49.9	55.3	55.6	58.1	66.8	78.5	72.0	60.5
Annual	54.9	58.2	62.3	61.4	70.0	72.9	80.5	59.0	70.0

The fact that this alternation of temperature may make a considerable difference in the maximum temperature the fungus can withstand was brought out in the discussion of the effects of temperature on the mycelial growth of the fungus. It was shown there that when exposed continuously to a temperature of 29.4° C. the fungus was killed in 9 days, but that it could live for 24 days or longer at 31.8° when exposed to this temperature for only 7 hours a day.

Mean relative humidity data for these localities given in Table III show that the relative humidity in San Francisco is much higher than in either San Jose or Los Angeles. These figures only approximately represent conditions as they exist in the Colma section. In this section fogs are very common, especially during July and August, and it is believed that the humidity is considerably higher here at times than in San Francisco. The figures given for Los Angeles are too high for certain of the cauliflower sections of Los Angeles County and too low for others, but they probably represent a fairly good average. Although reliable weather data can be obtained only in the fields where the crops are growing, these figures give a comparative idea of the temperature and humidity conditions in these three sections.

INCUBATION PERIOD

It was stated under the discussion of symptoms that the first evidence of infection was characterized by the appearance of small dark-colored spots one-half millimeter or less in diameter. In order to make sure that these minute spots were early stages of ringspot, and to get a better idea of the time required for the spots to develop, detailed observations were made on a number of plants. On January 9, 1923, three plants whose leaves had a considerable number of small spots were brought from the field and set in pots in the greenhouse. The exact time at which infection took place was not known but it was thought that this happened on January 3, when the foliage was known to have been wet all day and night and until noon the following day. The lower leaves of these plants were heavily infected, the largest lesions being about 1 mm. in diameter. On January 15 some of the largest spots had a diameter of 3 to 4 mm. Spots which on January 9 were mere points were from 3 to 5 mm. on January 20, and were 5 to 8 mm. in diameter on January 30, respectively. Plate 4, C, shows one of these leaves as it appeared on January 30. Isolations showed these spots to be due to the ringspot fungus. In another instance 15 plants similarly infected were brought into the greenhouse and observed. These showed that infection may become evident as very minute spots in from 3 to 6 days after inoculation, and that under favorable conditions the spots may reach a diameter of 4 to 5 mm., with pycnidia becoming evident in 21 days. It usually requires about a month from the time infection takes place for the development of mature pycnidia or perithecia. Some plants from the same lot as the 15 mentioned above were planted in the pathological garden near the greenhouse. The lesions on these plants developed much more slowly than on those in the greenhouse. It is possible that under conditions unfavorable for the development of the fungus several days may elapse after infection

takes place before it becomes evident, also that the lesions may require considerably more than a month to mature. This is also indicated in the inoculation experiments discussed above. For example, in one experiment inoculations were made with mycelium from pure culture on January 14, 1924, but infection was not evident until February 16. In most of the inoculation experiments from 20 to 35 days elapsed between the time the inoculations were made and the time when unmistakable evidence of infection was noted.

EFFECT OF THE FUNGUS ON THE HOST

The mycelium passes between and through the cells in all directions from the point of infection, forming a more or less circular dead spot which extends entirely through the leaf. After two to four weeks the mycelium forms globose masses of cells in the substomatal cavities on both the upper and lower sides of the leaf which develop into perithecia and pycnidia. Not only are the affected tissues of the plant killed, but the uninfected areas between spots often die as a result of cutting off their food supply. So, either directly or indirectly, the leaf may be so badly injured that it eventually drops from the plant.

RINGSPOT AS A FACTOR IN TRANSIT

Osmun and Anderson (*20*) found many yellow leaves on cauliflower which had been shipped from California to Boston. Apparently the yellowing of the foliage was attributed by them to the ringspot. Experiments were performed by the writer to determine to what extent the ringspot develops under cold-storage conditions which would approximate those found in a refrigerator car. Cauliflower heads surrounded with the usual crown of leaves, some diseased and some healthy, were held in cold-storage chambers or at room temperature for different periods. The heads with diseased leaves came from the Colma section; those free of disease came, necessarily, from the eastern side of the bay. In all cases heads were placed in cold storage as soon as possible after cutting. In some instances the leaves were quite badly spotted, while in others they had only a few spots. The data from one experiment of this nature are summarized in Table IV. These figures show that yellowing and dropping of the leaves of cauliflower takes place in storage regardless of the presence or absence of the ringspot fungus. The rate of yellowing varies with the temperature to which the cauliflower is exposed. After one week's exposure at 7° C., from 3 to 6 of the outer leaves were yellow and loose. All the leaves dropped from both the diseased and healthy plants at this temperature in two weeks or less. At 5° the yellowing was slower, an extra week elapsing before the leaves had all yellowed. Yellowing had just begun at 0° at the end of a month, when the final observations were made. This experiment shows that the rate of yellowing was not affected by the disease but varied with the temperature. Likewise, a spreading of the spots, or the origin of new spots, could not be detected in any case.

TABLE IV.—*Effect of ringspot on the yellowing and falling off of diseased and healthy leaves of cauliflower stored at different temperatures*

Source and condition of cauliflower	Temperature (°C.)	Number of heads	Condition of leaves after being in storage for—			
			1 week	2 weeks	3 weeks	4 weeks
San Francisco wholesale market; diseased.	7	9	3 to 6 outer leaves yellow and falling.	Leaves all yellow and falling; discarded.	----------	
Do	5	9	Some yellowing in 2 to 3 outer leaves.	Three-fourths of leaves yellow and falling.	All leaves yellow and loose; discarded.	
Do	3.5	9	No yellowing	Yellowing just starting.	One-half to three-fourths of leaves yellow and loose.	Practically all of leaves yellowed and fallen off.
Do	0	9	do	No yellowing	Leaves green and firmly attached.	Bottom leaf only starting to yellow.
Oakland wholesale market; healthy.	7	5	3 to 6 outer leaves yellow and falling.	Leaves all yellow and falling; discarded.	----------	
Do	5	5	Some yellowing in 2 to 3 outer leaves.	Three-fourths of leaves yellow and dropping.	All leaves yellow and loose; discarded.	
Do	3.5	5	No yellowing	Yellowing just starting.	One-half to three-fourths of leaves yellow and loose.	Practically all leaves yellowed and fallen off.
Do	0	5	do	No yellowing	No yellowing	Only bottom leaf starting to yellow.

In order to test these points on a larger scale, cauliflower with diseased and healthy leaves was obtained from the San Francisco and Oakland wholesale markets. One lot of heads from the San Francisco market had very badly diseased leaves, some of the lower ones already starting to yellow. Two other lots from the same source, one of cauliflower and the other of broccoli, had many lesions on their lower leaves, but these were small and the leaves green. The healthy plants from Oakland were fresh and in excellent condition. All of the heads were trimmed and packed in crates as for shipment, and stored under temperatures of 2.2°, 1.4°, and 0° C. in a large commercial cold-storage plant in San Francisco. The condition of the plants at different intervals are given in Table V. The data given in this table show that here again no difference could be detected in the rate of yellowing and defoliation which could be attributed to the disease. Although the badly diseased lot showed slightly more yellowing and defoliation than the others, this cauliflower was not so fresh and the lower leaves had started to turn yellow before the experiment was begun.

Under the conditions of these experiments it was impossible to keep cauliflower at a temperature much above 0° C. for longer than three weeks without losing some of the leaves. On the other hand, considerable yellowing and defoliation occurred in a week at 5° and 7° C. However, it must be remembered that the conditions of a refrigerator car were not reproduced here. No account was taken of the humidity or any other factors except temperature and disease. These experiments were designed to determine whether the fungus

was responsible for any of the yellowing and defoliation which takes place under cold-storage conditions. They have shown that the yellowing takes place with equal rapidity in both the diseased and healthy cauliflower under commercial cold-storage conditions. Presumably the same correlation would hold under transit condition.

TABLE V.—*Effect of ringspot on the yellowing and falling off of leaves of cauliflower stored at different temperatures*

Source and condition of cauliflower	Temperature (°C.)	Number of heads	Condition of leaves after being in storage for—			
			1 week	2 weeks	3 weeks	4 weeks
San Francisco market; badly diseased; few leaves starting to yellow.	2.2	20	Little change.	2 to 4 outer leaves yellowing.	One-fourth to one-half of leaves yellowing and falling.	2 per cent of heads defoliated; leaves greenish yellow; other heads with some green leaves.
Do	1.4	20	do	Slight increase in yellowing.	do	Do.
Do	0	20	do	do	An occasional leaf yellow.	Few heads entirely defoliated; some with 2 to 6 lower leaves fallen off; many with leaves still green and firmly attached.
San Francisco market; leaves diseased but fresh and green; cauliflower.	2.2	27	do	Yellowing and loosening of bottom leaves starting.	One-fourth to one-half leaves yellowing and falling.	Most heads defoliated; few with some green leaves.
Do	1.4	27	do	Slight yellowing	do	Do.
Do	0	27	do	No yellowing	An occasional leaf yellowing.	Some heads with all leaves green and firmly attached; others with 5 to 6 lower leaves yellowing.
San Francisco market; broccoli	2.2	10	do	Few leaves yellowing and loosening.	One-fourth to one-half of leaves yellowing and loosening.	Most of heads defoliated; few with some green leaves.
Do	1.4	10	do	Yellowing just starting.	do	Do.
Do	0	10	do	No yellowing	An occasional leaf yellowing.	Some heads with all leaves green and firmly attached; others with 2 to 4 leaves yellowing and loose.
Oakland market; healthy broccoli.	2.2	20	do	Few leaves yellowing and loosening.	One-fourth to one-half of leaves yellowing and falling.	Most heads defoliated; few with some green leaves.
Do	1.4	20	do	Yellowing starting.	do	Do.
Do	0	20	do	No yellowing	An occasional leaf yellowing.	Some heads with all leaves green and firmly attached; some heads with 5 to 6 leaves loose and yellow.

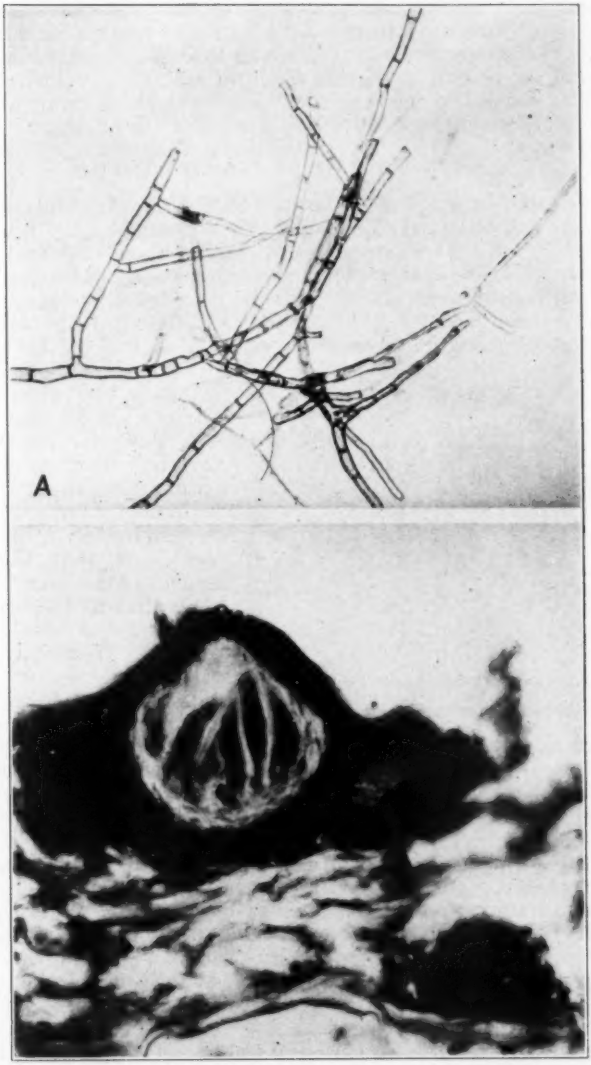
ENLARGEMENT OF THE RINGSPOT LESIONS

Having been unable to observe any spread of the disease under the conditions of the above experiments, an effort was made to determine whether the spots actually enlarged after the plants were cut. Leaves both detached and attached to the plants, and with lesions of different sizes, were used. A number of the lesions were outlined with India ink and their diameters were measured before the leaves were placed in storage. The leaves were marked and the spots were numbered, so that the same spots could be measured later and their diameters compared with the original measurements. In other cases the spots were ringed, but were not measured. Temperatures of 0°, 3.5°, 5.7°, and 18° to 22° C. were used. The humidity of the chambers ranged from 70 to 87 per cent. Likewise, some leaves were placed in moist chambers which were held at the different temperatures. The spots enlarged under all of the conditions tried. The diameters of the spots were about 1 to 3 mm. greater after a week at 18° to 22° than at the start. The spots increased in diameter somewhat more slowly at the lower temperatures, but a slight increase was apparent even at 0° in about two weeks. Not only was the enlargement of the spots evident, but also fruiting bodies began to appear outside of the ink ring in 12 days at 9° and 18° to 22°. Although actual transit conditions were not reproduced, the rate of enlargement of these spots under the conditions of these experiments indicates that under transit conditions, and in the time it would take for the cauliflower to reach the eastern markets, there would be little, if any, increase in their size which would be noticeable to the casual observer.

MORPHOLOGY

The ascospores germinate readily in water, forming a mycelium about 2 to 4.5 μ in diameter. The mycelium is hyaline at first but turns darker with age, the color ultimately attained depending upon the medium upon which it is growing. The threads may branch profusely and become closely septate, forming a sclerotiumlike growth, or they may branch sparingly and have a few septa, the variation depending upon the food, moisture, and temperature conditions. In the host the hyphae are usually dark-colored, much branched, and closely septate, cells measuring from 5 to 30 μ in length being common (pl. 5, A). The young cells are filled with densely granular protoplasm, which frequently contains many oil drops. The cells of two parallel hyphal strands are not uncommonly united by a short thread growing perpendicularly to both.

Globose masses of hyphae form in the substomatal cavities from which the pycnidia and perithecia are differentiated. These develop singly and without a stroma. The pycnidia are generally globose and dark in color. They vary somewhat in diameter, but are usually from 75 to 100 μ. The pycnospores are nonseptate, cylindrical, bacilloid, straight or only slightly curved and obtuse at the ends. They are thin-walled, hyaline, and measure from 1 to 2.75×3 to 7.5 μ (fig. 2, B). At first the perithecia are nearly globose, with a short neck, but later they become flask-shaped to pyriform. They are dark colored, and measure 80 to 112×90 to 125 μ (pl. 5, B). The asci develop in a tuft at the base of the perithecium. These



A.—Pure cultures of *Mycosphaerella brassicicola*. Cultures Nos. 24-c and 17 are on potato agar, plus 1 per cent dextrose. Culture No. 17 shows the white marginal growth often seen in young cultures. A culture on Czapek's agar is shown in tube No. 11. Note the peculiar feathery growth somewhat resembling the irregular lesions illustrated in Plate 1. The culture in tube 24-c developed from a transfer from the feathery growth. × ¾

B.—Numerous colonies of *Mycosphaerella brassicicola* 9 days old, growing on potato agar in a Petri dish. A piece of the mycelial mat from a pure culture was crushed in a tube of melted agar, which was then poured into the Petri dish. × ¾

do not develop simultaneously, so that asci in all stages of development may be found in the same perithecium. The asci are cylindrical-oblong, measure 8 to 12×40 to 50 μ, and contain eight 2-celled spores, which are eventually discharged through the ostiolum. The ascospores are oblong or slightly fusiform in shape, hyaline, straight or slightly curved, somewhat constricted at the septum, blunt at the ends, and measure 3.5 to 5.5×15 to 25 μ (fig. 2, A).

GROWTH OF THE FUNGUS ON CULTURE MEDIA

The ringspot fungus was transferred to the following culture media and its growth observed: Beef agar (bacto nutrient agar) plus 1 per cent and 2 per cent dextrose, potato agar alone and with 2 per cent dextrose, prune agar, carrot agar, cauliflower agar, Czapek's agar, corn meal, Coons' medium (*10*), *Melilotus alba* stems, cooked and raw cauliflower leaves, and cauliflower juice. A fair growth took place on all but the raw cauliflower. However, a slightly better growth developed on the beef agar than on the other media. Growth on all the media was slow; for example, a mycelial mat, circular in outline and about 0.5 cm. in diameter, was 20 days in forming on a beef-agar slant. At that time the hyphal mat was nearly black, but had a brownish or olivaceous tint similar to that frequently seen in some species of Alternaria. The colony had a narrow margin of gray. The surface of the felt lay in folds or wrinkles, extending from the margin toward the center. A few pycnidia containing apparently mature spores had developed. Plate 6, A, B, shows the general character of the colonies formed on agar. The growth on agar was usually circular or elliptical in outline and spread over comparatively little of the surface; but on cooked cabbage leaves, Melilotus stems, rice, and corn meal it formed a thin dark or grayish covering. The color of the mycelium varied considerably both with the medium and the age of the culture. In some cases it was pure white, as, for example, the new growth at the margin or over the surface of a planting when growing on filter paper saturated with Coons' medium, on the surface of old cultures on cooked cauliflower leaves, and on some old cultures on potato agar where new growth had started after the culture had remained dormant for a period of time. The fungus growing on prune and carrot agar was white on the surface but quite dark where it was in contact with the medium. A 2-months-old culture growing submerged in Coons' medium was olivaceous in color. Most of the agars were slightly darkened by the fungus. Prune and carrot agars proved to be good media for mycelial growth but not for fruiting. Of all the media tried, beef and potato agars plus 1 or 2 per cent dextrose, and cooked cauliflower leaves, proved to be the best

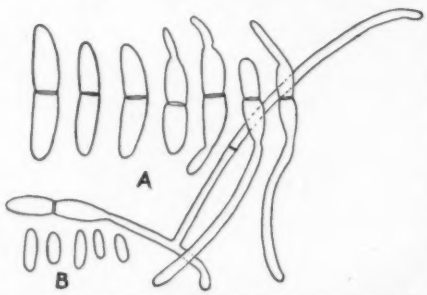
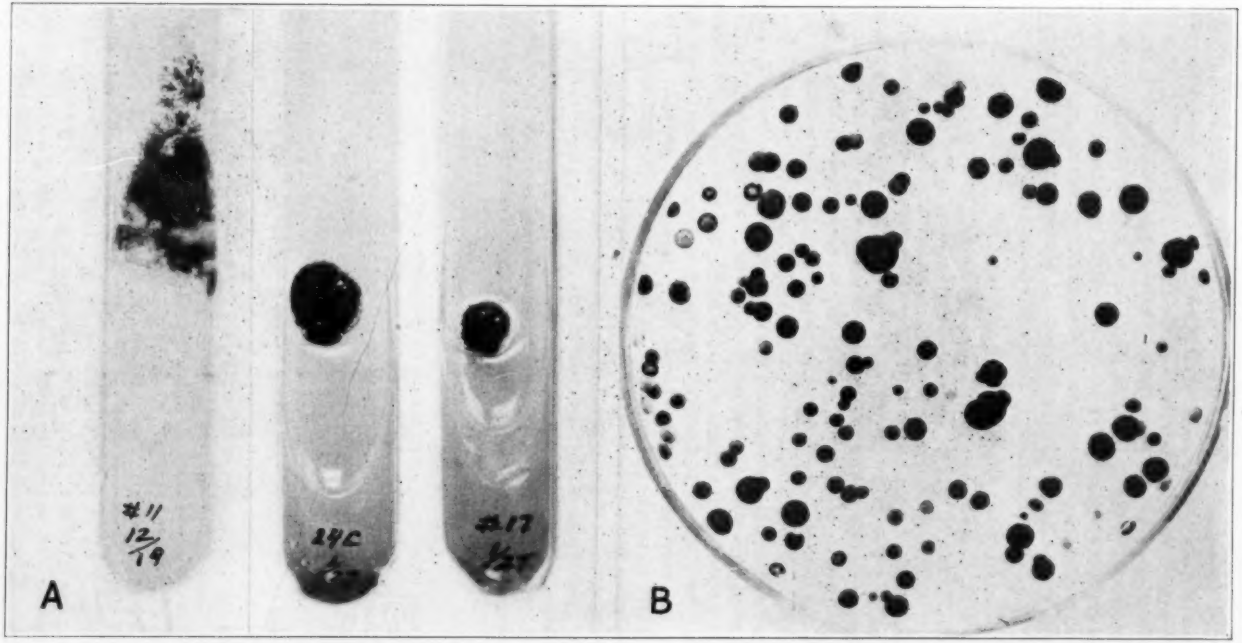


FIG. 2.—(A) Ascospores of *Mycosphaerella brassicicola*. Different stages of germination are also illustrated. × 1500. (B) Pycnospores of *Mycosphaerella brassicicola*. × 750



A.—Hyphae of *Mycosphaerella brassicicola* from a pure culture. × 600
B.—Photomicrograph of a perithecium of *Mycosphaerella brassicicola* developed on a cauliflower leaf, showing asci with spores. × 500

for both mycelial growth and fruiting. These media fulfilled the needs admirably so far as hyphal growth was concerned, but very few spores were formed on any of them.

A peculiar type of growth was noticed occasionally in cultures. This is shown in the tube 11 in Plate 6, A. When small pieces of this mycelium were transferred to fresh media growth took place in the normal manner. This variation seemed to occur on dry media, especially on Czapek's agar. Some of the lesions in the leaves also showed a similar irregular, feathery character, as previously described.

CONTROL

SEED-BED SANITATION

The value of certain control measures was tested in the Colma section. It was pointed out under the discussion of the inoculation experiments that young plants in the seed bed could become infected from the pieces of infected seed pods or old leaves on the ground beneath the plants. No doubt the fungus can live for a time in the soil also. It is important, therefore, that precautions be taken to eliminate all of the old plants from the new seed bed and vicinity. Pieces of infected seed pods should not be allowed to reach the seed bed along with the seed. The location of the seed bed should be changed frequently or the old soil sterilized or replaced with soil from a field where diseased cruciferous crops have not been grown. It is also important to locate the seed bed as far as possible from any infected fields and to have it protected from the prevailing winds which might carry spores for a considerable distance. All old plants growing in the neighborhood of the beds should be removed, as these may serve as sources of infection. Likewise, fields on which diseased plants have been grown should be plowed as soon as possible after the crop has been harvested. These serve as a source of infection for the new crop.

SEED TREATMENT

Several attempts were made to determine the effectiveness of seed treatment, but owing to unfavorable weather conditions no disease developed on either the control plots or those sown with treated seed. It hardly seems worth while, therefore, to record the details of these trials.

Although the experiments which were carried out to demonstrate that the fungus could be killed when in or on the seeds failed to yield results, the data given above under the discussion of the susceptibility of the fungus to heat may shed some light on the possibilities of controlling this fungus by the application of heat. It was pointed out under that discussion that the mycelium of the ringspot fungus was killed by an exposure of 5, 10, or 15 minutes to a temperature of 55 to 56° C. or of 10, 20, or 30 minutes to 50 to 51° C. Chupp (*7*) reports a 100 per cent killing of Alternaria in the seeds of cabbage by treating them with hot water at 56° for 10 minutes. He also states that Walker (*25*) found that treating cabbage seed with hot water at 55° for 10 minutes completely eliminated *Alternaria brassicae*. Weimer (*27*) showed that the optimum and maximum temperatures for the growth of *Alternaria brassicae* in culture are between 25° and 27° and 38°, respectively. These are several degrees higher in each

case than the corresponding temperatures for *Mycosphaerella brassicicola*. It is logical, therefore, to suppose that *M. brassicicola*, being considerably more sensitive to heat than is *A. brassicae*, would be killed by the seed treatment recommended for the latter, namely, by immersing in water at a temperature of 55° for 10 minutes or 50° for 30 minutes.

SPRAYING

As already pointed out, the cauliflower fields in the Colma section are so numerous and so close together that one serves as a source of infection for another. The seed beds are almost always located in or near a field of cabbage or cauliflower more or less heavily infected with ringspot; hence seed-bed sanitation and seed treatment are largely nullified. Experiments in which disease-free plants grown at Berkeley were planted at Colma showed the futility of any control method based on the production of disease-free plants for this locality. In these tests the healthy plants very soon were as badly affected with ringspot as were the plants grown at Colma and exposed to infection in the seed beds. The production of clean plants would no doubt be a very helpful method of control in Oregon, where the fields are far apart and the disease has not become well established.

Having shown the ineffectiveness of these methods of control under Colma conditions, spraying and dusting were tried. Spraying and dusting experiments were carried out during the winters of 1922–23 and 1923–24.

Spray Material Used

Homemade Bordeaux mixture, 4–4–50, with and without a spreader, was used both seasons. The first season (1922–23) Kayso and liquid whale-oil resin soap, both at the rate of 4 pounds to 50 gallons of spray, were added to the Bordeaux as spreaders. In 1923–24 only the soap was used, inasmuch as this had proved to be the most satisfactory the previous year. Copper-lime dust, containing 20 parts of copper and 80 parts of lime, and a powdered Bordeaux mixture, containing 21.82 per cent of copper, were applied in the dust form to certain plots in 1922–23. Owing to the injury caused by the 20–80 copper-lime dust, a 15–85 preparation was used the following year. In addition, a dry Bordeaux mixture containing 12.5 per cent copper was used in dry form, and barium tetrasulphide (1 pound to 25 gallons of water) was applied in solution in 1923–24. Likewise, since the 4–4–50 Bordeaux caused injury in 1922–23, 4–3–50 and 2–2–50 were tried the following year, both with and without soap added.

The wet sprays were applied with a 4-gallon compressed-air sprayer. A small hand duster was used for the dusts. Every effort was made to keep the pressure in the spray tank as high as possible, so that a fine spray would be obtained. The dusts were applied for the most part early in the morning while the leaves were wet. The plants were covered as thoroughly as possible under the conditions. The tendency of the liquid sprays to form large drops and roll off the leaves was lessened somewhat when soap was added. A much better covering of the leaves would have been possible with a power sprayer and the higher pressure which would then have been available, but such a sprayer could not be used on account of the texture of the soil and the contour of the land. A fairly good coating of dust was possible with the equipment used.

In the 1923–24 experiment, plots of about three-twentieths of an acre each were used, in duplicate. The plots in 1922–23 were about half this size. An unsprayed control equal in size to the sprayed plots was left between each of the latter. Seven applications were made in 1922–23 and six in 1923–24, except for a few rows in the 1922–23 plot which were sprayed only four times.

RESULTS OF SPRAY TESTS

Owing to the fact that there was so little of the disease present in the 1923–24 spray plot, no conclusions could be drawn regarding the effectiveness of the spray materials. The 1922–23 spray tests showed very slight if any control. The ringspot developed abundantly on the sprayed and dusted plots, as well as on the checks. Careful study of the number of spots on the leaves of the plants in the various plots showed a slight difference in favor of the sprayed plots. However, the sprayed and dusted plants were injured to such an extent that many of the lower leaves, which were also the most susceptible, fell off. The fact that the sprayed and dusted plants had a smaller number of susceptible leaves may account for the difference in the number of spots on these plants. The same conditions existed in all of the plots; hence no one of the sprays or dusts used was shown to be more efficient than another. Likewise, the plots sprayed and dusted seven times were as badly diseased as those given four applications.

SPRAY INJURY

Injury in the 1922–23 plot was first noted after the third application. It was evident in some cases by the crinkling of the leaves and in others by a burning usually along the margins or in places where the spray had collected. The lower leaves turned yellow and fell off prematurely, resulting in a partial defoliation and a consequent stunting of the plants. The injury was evident in about the same degree in all sprayed and dusted plots. No spray injury was apparent in the plots in 1923–24 with any of the materials used. The writer is unable to account for the fact that all of the materials used in 1922–23 but none of those used in 1923–24 caused injury, unless it was due to the higher relative humidity existing during the former season.

Sherbakoff (*24*), Kirk (*17*), Fawcett (*13*), Higgins (*16*), Harter and Jones (*15*), and others recommend spraying cabbage with Bordeaux mixture for the control of leafspots. None of these workers mentions any injury to this host resulting from the use of this material. Clayton (*8*), however, reports serious loss due to spraying cauliflower with Bordeaux.

EFFECTIVENESS OF SPRAY MATERIALS

Since spraying failed to give any appreciable control of the ringspot, it was thought that possibly the spores of the causal fugus were unusually resistant to spray mixtures. To determine this point, tests were made in which cover-slips (4 for each preparation) were sprayed or dusted with the materials used in 1923–24. The cover-slips were then placed over van Tieghem cells with bits of cauliflower tissue containing perithecia floating on water as in previously reported experiments. The discharged ascospores were caught and

held in the water condensed on the under surface of the cover-slips. Clean cover-slips placed over van Tieghem cells, as described above for the sprayed cover-slips, served as controls. The preparations were held at 15–20° C. An examination of the controls after 20 hours showed that an abundance of spores had been caught and that 98 per cent of them had germinated and had formed germ tubes 0.5 to 3 times as long as the spores. Cover-slips sprayed with the following preparations had caught an abundance of spores, none of which had germinated after several days: Bordeaux 2–2–50, with and without soap; Bordeaux 4–3–50; Bordeaux 4–4–50, with and without soap; copper-lime dust (15–85); and Bordeaux dust (12.5 per cent copper). This experiment shows that the failure to control the leafspot was not due to ineffectiveness of the spray materials, but rather was probably due, at least in part, to the failure to get the leaves thoroughly covered. As mentioned above, the spray materials did not spread satisfactorily, even with Kayso or soap added, and a perfect covering of both sides of the leaves was impossible. Spraying is therefore considered impracticable under Colma conditions, but it may prove of value elsewhere.

VARIETAL RESISTANCE

Careful observations were made throughout the two seasons for plants which might show more or less resistance to the disease. Occasionally a plant was found which was not quite as heavily infected as its neighbors, but never was one found sufficiently free of spots to make it appear to possess any high degree of resistance. In order to determine whether any available varieties of cauliflower were resistant, a number were tested during the season of 1923–24.

The plants used in this experiment were grown at Berkeley from seed obtained from two commercial seed houses, one in San Francisco and the other in Reading, England. Seed of a locally developed strain of black broccoli was supplied by H. A. Jones, of the University of California. Ten plants of each variety were set in each of two fields at Colma. One of the fields was about a quarter of a mile from the ocean, while the other was 4 or 5 miles inland.

The varieties used were referred to by numbers, since in several cases the names were not supplied. Those the names of which were known are:

Varieties from the San Francisco seed house:
- Cauliflower—
  - Early Snowball.
  - Dry Weather.
  - Autumn Giant.
- Broccoli—
  - Large White French.
  - St. Valentine.

Varieties from the Reading (England) seed house:
- Cauliflower—
  - Sutton's Autumn Mammoth.
  - Sutton's Magnum Bonum.
  - Sutton's Universal.
  - Sutton's Purity.
  - Sutton's Early Giant.
  - Sutton's First Crop.
  - Sutton's Improved Large Erfurt.

Varieties from the Reading (England) seed house—Continued.
- Broccoli—
  - Sutton's Favorite.
  - Sutton's Autumn Protecting.
  - Sutton's Improved White Sprouting.
  - Sutton's Winter Mammoth.
  - Sutton's Safe Guard Protecting.
  - Sutton's Standwell.
  - Sutton's Purple Sprouting.
  - Sutton's Peerless (Superb Early White).
  - Sutton's Peerless (Snow White).
  - Sutton's Whitsuntide.
  - Sutton's Bouquet.

The season of 1923–24 was unusually dry, and little leafspot developed. However, before the plants matured sufficient disease was present on the lower leaves to show that none were immune and, as nearly as could be judged, all were about equally susceptible. The strain of black broccoli grown on the ranch on which the plot was situated proved to be as good under the conditions of this experiment, both from the standpoint of production and of disease resistance, as any of those tested.

Most of the growers in this locality raise their own seed. Some of them select their best plants each year, transplant these in an isolated corner of the ranch, and gather their seed from them. By this method some very fine strains have been developed. These when planted on fertile soil and well cultivated and irrigated often produce an excellent product in spite of the ring spot. It seems that selection for hardiness, combined with selection of plants with the smallest number of spots, coupled with the best horticultural practices, is the best method of combating the disease in this section.

CROP ROTATION

A considerable acreage of cruciferous crops is grown the year round in the Colma section, which keeps the disease going in virulent form the entire year. No doubt the disease could be checked to some extent if the crucifers were limited strictly to a winter crop and every sanitary measure practiced to eliminate plants which might carry the disease over from one season to the next. In Oregon, where a different type of agriculture is in vogue, crop rotation will no doubt prove of value in controlling this disease. A cruciferous crop should not follow a crop which shows any ring spot for at least two years and preferably not for three or four years. Any noncruciferous crop can be used in the rotation.

SUMMARY

A leaf spot, known as ring spot, due to *Mycosphaerella brassicicola*, causes considerable loss to cruciferous crops in the San Francisco Bay region and in Oregon, especially during wet seasons.

White broccoli, black broccoli, cauliflower, Brussels sprouts, cabbage, yellow turnip, and kale are susceptible. The degree of susceptibility decreases in the order named. The disease was not found on white turnip or radish.

The symptoms of the disease on different hosts are described and illustrated.

The causal fungus has been isolated and its pathogenicity proved.

In California the perithecial is more abundant than the pycnidial stage during the greater part of the cauliflower season, and the ascospores are thought to be responsible for a major part of the infection. The ascospores are forcibly ejected from the perithecium, after which they are scattered broadcast by the wind. The disease may also be disseminated with the seedlings, the seed, the seed pods, the leaves, and possibly in the soil.

The fungus enters the host through the stomata. Leaves having 300 or fewer stomata per square millimeter of leaf surface are susceptible, while those with more than this number are resistant.

The optimum temperature for spore germination and mycelial growth lies between 15° and 22° C. Some growth will take place at 0°. The fungus when growing on agar can withstand a temperature of 32° to 33° for 3 days, but it is killed in 4 days at this temperature. It is killed in less than 15 minutes at 55° to 56°.

Infection becomes evident as minute dark spots in 3 to 6 days. Under favorable conditions the spots reach a diameter of 4 to 5 mm., with pycnidia becoming evident in 21 days. Approximately a month is necessary for the development of mature pycnospores and ascospores.

The ring-spot fungus has been grown on several different culture media. It grows well on most of the media used. Beef and potato agars, with 1 or 2 per cent dextrose added, and cooked cauliflower leaves, were the most satisfactory of the media used. Prune and carrot agars proved to be good for mycelial growth but not for fruiting. Very few spores were formed on any of the media used.

Cold-storage tests indicate that the spots do not spread rapidly enough to make them a serious factor in deterioration of cauliflower in transit. The yellowing and dropping of the foliage of cauliflower in transit is largely due to physiological changes that are in no way associated with ring spot.

The value of different control measures is discussed in detail. Control measures based on the production of disease-free plants by seed-bed sanitation and seed treatment are of little value in the Colma section under the system of agriculture now practiced there, but no doubt such would be helpful in Oregon, where a different system of crop rotation is in vogue. The seed should be freed of all débris and then treated by immersing in water for 10 minutes at 55° or for 30 minutes at 50° C. Spraying and dusting not only proved to be ineffective but were injurious to the plants. Of the cauliflower and broccoli varieties tested none was found to be more resistant than those grown at Colma. Careful selection for hardiness, together with selection of plants with the smallest of spots for seed, coupled with the best horticultural practices, seems to be the best method for controlling this disease under conditions existing in the Colma section.

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RELATION OF TEMPERATURE TO SPORE GERMINATION AND GROWTH OF UROCYSTIS CEPULAE[1]

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INTRODUCTION

In a previous paper (*21*) [2] the relation of soil temperature and soil moisture to infection by the onion-smut fungus (*Urocystis cepulae* Frost) was considered. As the onion seed germinates the cotyledon, functioning as the first leaf, is readily invaded by the parasite until it becomes mature. This is usually a period of two to three weeks, following which the mature cotyledon and the successively maturing true leaves serve as protecting sheaths which exclude further infection. The soil environment during this susceptible period is therefore a critical one in determining the extent of infection.

Invasion by the smut organism occurred at temperatures as low as those at which germination and growth of the host ordinarily takes place (10° to 12° C.), but whether or not there was an extremely low point at which the host might escape the parasite was not determined. Infection occurred regularly at 25°C., but above that point the amount of disease rapidly declined, until at a constant temperature of 29° it was completely inhibited. During these studies the question arose as to the exact nature of this temperature inhibition, i. e., whether it was a case of inhibition of the parasite or of stimulation of greater resistance on the part of the host, or both. With the methods in use at that time it was impossible to separate these factors. It has become necessary, therefore, to consider the relation of environment to the development of the parasite as apart from the host. This paper embodies results of a study of the effect of temperature on the germination and growth of *Urocystis cepulae* and its bearing upon infection.

PREVIOUS WORK ON RELATION OF TEMPERATURE TO SMUT SPORE GERMINATION AND INFECTION

Considerable attention has been given by previous workers to the temperature relations of the smut fungi, especially those attacking the cereal crops. Much of the earlier literature has been amply reviewed by Jones (*10*). Several more recent contributions are of special interest. In a critical study of corn smut (*Ustilago zeae* (Beckm.) Unger) Jones (*11*) found that spore germination occurred throughout a temperature range of 8° to 38° C., the optimum lying between 26° and 35°. In another study the same writer (*10*) found the range of spore germination of *Ustilago avenae* (Pers.) Jens. to extend from 4° to 34°, with the optimum at 15° to 28°.

[1] Received for publication Jan. 26, 1925; issued February, 1926. This investigation was carried on in cooperation with the Department of Plant Pathology, University of Wisconsin.

[2] Reference is made by number (italic) to "Literature cited," p. 145.

Numerous recent infection studies on the relation of environment to the development of cereal smuts bring together evidence that infection takes place over a comparatively wide range, but in general the amount of infection is found to decrease at the extremes. Involved in the environmental complex, however, are the interdependent relations of soil temperature, soil moisture, and soil reaction, all of which should be considered in the final interpretation of the influence of any single factor. Working with the loose smut of oats (*Ustilago avenae*), Bartholomew and Jones (*3*) showed that low temperatures (8° to 12° C.) during the susceptible period of the host might reduce infection somewhat, but the reduction was more marked at the upper limit of the range of spore germination. When a soil temperature of 31° to 32° was combined with high soil moisture, the inhibition of the fungus was complete. In a parallel study with covered smut of oats (*Ustilago levis* (K. and S.) Magn.), Reed and Faris (*17*) found this organism to correspond very closely with *Ustilago avenae* in behavior.

The covered smut (*Sphacelotheca sorghi* (Link) Clint.) and loose smut (*Sphacelotheca cruenta* (Kühn) Potter) of sorghums were studied by the same workers (*16*). The two organisms were found to behave very similarly, infection occurring from 12° to 37.5° C. There was a reduction of the amount of infection at the extremes, but here again the degree of reduction was influenced by soil moisture. Faris (*6*), working with covered smut of barley (*Ustilago hordei* (Pers.) K. and S.), found infection to occur over a range of 5° to 30°, with a decided reduction in the amount of disease as the temperature approached the two extremes.

The influence of temperature upon flag smut of wheat (*Urocystis tritici* Koern.) has been studied recently by Noble (*14*). Spores presoaked in water for some days germinated at lower and at higher temperatures than those placed directly at fixed temperatures without presoaking. Such presoaked spores lost their viability more readily at the higher temperatures than did the spores which were not presoaked. Germination occurred at as low as 5° and as high as 32° C. Approximately the same percentage of spores germinated throughout the range from 18° to 27°, but the maximum growth of germ tubes occurred at 24°. Incubation at constant temperatures above 25° proved to be detrimental to growth following germination. In controlled experiments (*15*) optimum infection occurred at 19° to 21°, the amount was decidedly reduced at 24° to 26°, while at 29° to 31° it was practically inhibited. Tisdale, Dungan, and Leighty (*20*) in successive planting experiments in Illinois found that in seedings made after November 1 there was marked reduction in the amount of disease, due presumably to inhibitive effect of low soil temperature.

METHODS OF EXPERIMENTATION

The current conception of the mode of spore germination of *Urocystis cepulae* has been modified by the recent work by Anderson (*1*). He secured germination readily as soon as spores became mature; a period of rest was unnecessary. Most satisfactory germination resulted from placing spores aseptically upon the surface of agar plates. Anderson shows that, contrary to previously held ideas,

there is no true promycelium produced upon germination of the chlamydospores, but instead a rounded vesicle with a diameter usually somewhat less than that of the central spore. From this vesicle hyphal branches are formed which extend and branch into a mycelial thallus without the expected production of secondary spores or sporidia. Somewhat later certain portions of the mycelium become many-septate and break up into detached cells. These "hyphal fragments" are germinable, and Anderson suggests that they may assume some of the functions of true sporidia. The present writers' findings agree with those of Anderson as to the morphology of the fungus as it grows on agar surfaces.

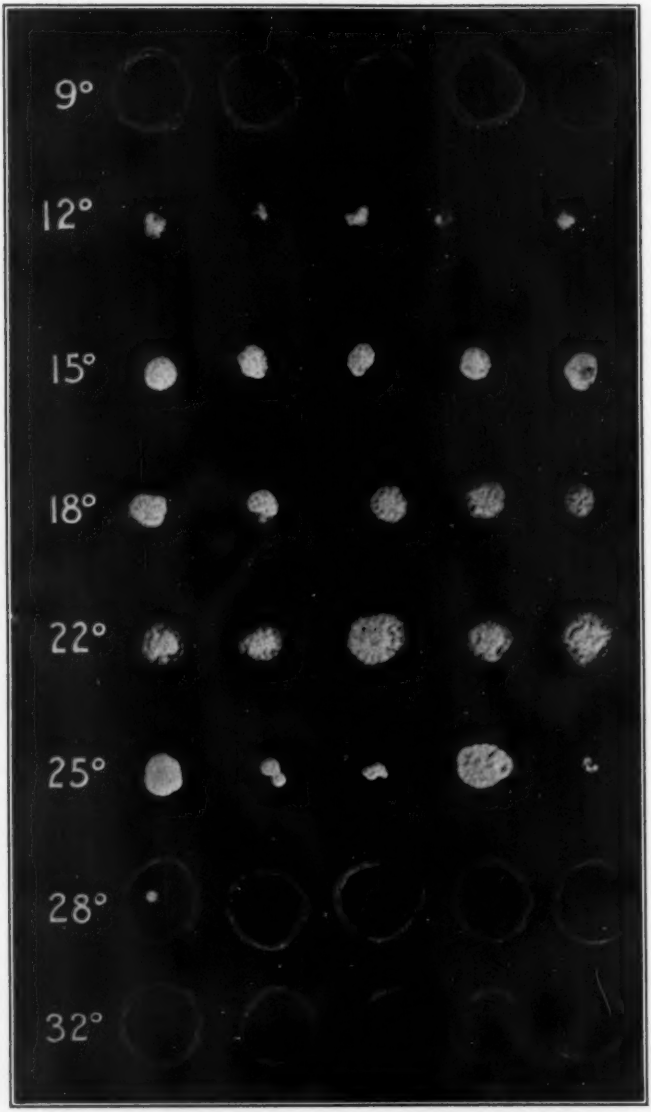
For germination studies, plates were prepared by first transferring spores aseptically from unbroken host lesions to tubes of onion decoction. Drops of this suspension were then transferred to the surface of plates of water agar or onion agar. Such plates were then transferred to constant temperatures of the desired range, and germination was estimated by counting several microscopic fields from each plate. Pure cultures of the fungus were obtained from such plates. From these cultures suspensions of hyphal fragments were readily secured. Drops of such suspensions were transferred to agar plates and germination at various temperatures determined, as in the case of chlamydospores. The rate of growth of hyphae was estimated by measuring the length of a certain number of germinating fragments in each plate at a given interval, and also by photographing the gross colonies from single drops of the hyphal-fragment suspension after a given period. (See pl. 1.)

EXPERIMENTAL RESULTS

GERMINATION OF CHLAMYDOSPORES

The process of chlamydospore germination is comparatively slow. Very little occurred before 5 days, and reliable comparative counts were not usually possible before 10 days. In some of the experiments increase in germination continued at certain temperatures even after two weeks. The tests were repeated a large number of times with concordant results in general, although there are occasional discrepancies. Some of the experimental results are given in Table I, and a representative graph is given in Figure 1. It will be seen that there is only slight germination at 9° C. This is slightly above the minimum for onion-seed germination and growth. It would appear that the fungus can develop at nearly as low a temperature as the host.

In the majority of cases the highest percentage of germination occurred at about 15° C. There were only slight decreases in germination, however, as high as 22°. The most rapid germination also occurred within this range. At 25° some detrimental effects of temperature are apparent in the reduced percentage of germination. Above this point there is a gradual but marked decline in germination and a decided retardation of growth of the hyphae. The extreme maximum is slightly above 32°. In many cases no germination was noted at 30° or above; in a few cases slight germination occurred, but the hyphae, in such cases, grew very little, and it is doubtful whether they would function in infection.



Plantings of distilled-water suspension of hyphal fragments were made on plates of onion agar. The plates were then placed in a series of constant-temperature incubator on February 22 (1924) and left until March 8. Resulting colonies from each temperature were then photographed

The white circles indicate no macroscopically visible growth. There is no evidence of growth at 9° C. There were small colonies at 12°; good growth at 15°, 18°, and 22°. There was evidence of retardation at 25°; very meager growth at 28°; and none at 32°. Note the prominent rugosity of the colonies at 22°. Rugosity evident to less degree at 15°, 18°, and 25°. Rugosity evident to less degree at 15° ,18°, and 25° ,while at 12° and 28° the colonies have a velvety appearance. × 6/10

The temperature range of the germination of chalmydospores is very close to that for germination of onion seeds. The optimum germination of the smut is to be expected in a comparatively cool soil, i. e., 13° to 20° C. Soil temperatures as they increase above 25° become more unfavorable. Although some germination may occur up to 32°, the growth of the hyphae, even on favorable media, is so scanty above 28° that one would expect it to advance very slightly in the soil in competition with the other soil organisms.

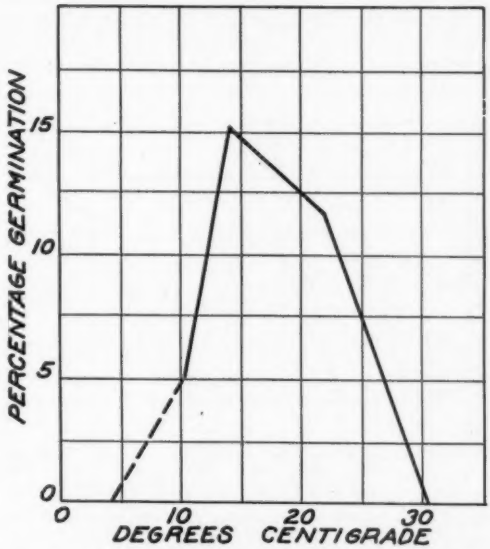


FIG. 1.—Relation of temperature to the germination of chlamydospores of *Urocystis cepulae*. (See experiment 3 in Table I)

TABLE I.—*Relation of temperature to germination of chlamydospores of Urocystis cepulae*

Experiment No.	Medium	Average temperature (° C.)	Estimated percentage of germination at various intervals			
			10 to 14 days		25 to 28 days	
			Spores counted	Per cent germinating	Spores counted	Per cent germinating
1	Suspension in onion decoction on onion agar substrate.	4.0	1,012	0.0	695	3.5
		9.0	857	0.2	674	28.5
		15.5	746	2.4	494	22.5
		20.0	262	19.1	.....	.....
		25.5	630	8.6	.....	.....
		27.0	490	7.4	.....	.....
		32.0	382	2.8	.....	.....
2	Suspension in onion decoction on water agar substrate.	9.0	1,228	0.0	.....	.....
		13.5	468	47.5	.....	.....
		23.5	305	17.3	.....	.....
3	do	10.5	298	5.3	.....	.....
		14.0	294	15.2	.....	.....
		22.0	279	11.6	.....	.....
		30.5	321	0.0	.....	.....
4	do	22.0	373	13.0	.....	.....
		26.0	168	9.5	.....	.....
		30.5	429	1.7	.....	.....

GERMINATION OF HYPHAL FRAGMENTS

The hyphal fragments germinate very readily. Final results on germination were obtained within a week. The data from a representative experiment are given in Table II, and shown graphically in Figure 2. No germination occurred at 4° C.; a fair amount occurred at 9°. The optimum, at about 15°, is close to that of chlamydospore germination. There is distinctly less germination at 20°, while the temperature of 25° is still more detrimental. Above 25° there is a very abrupt check in germination, which agrees closely with the sudden decline in infection at these temperatures. The temperature relations of the hyphal fragments are very close to those of the chlamydospores. The former become slightly more active at 9°, but less so above 25°.

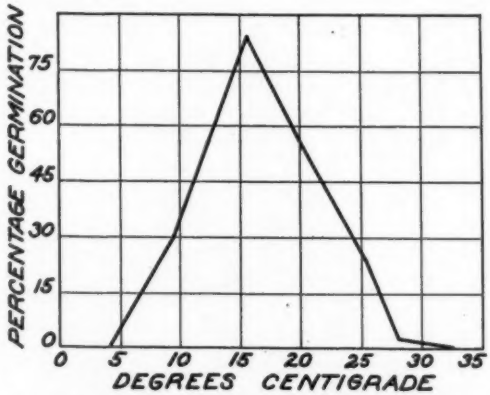


FIG. 2.—Relation of temperature to the germination of hyphal fragments of *Urocystis cepulae*. (See Table II)

TABLE II.—*Relation of temperature to germination of hyphal fragments of Urocystis cepulae*

Average temperature	Percentage of germination at various intervals	
	3 days	6 to 7 days
° C.		
4.0	0.0	0.0
9.0	4.3	27.9
15.5	90.7	84.0
20.0	32.9	55.6
25.5	26.4	22.1
28.0	3.0	2.7
32.0	0.0	0.0

VEGETATIVE GROWTH

The rate of growth of the mycelium was studied by measuring the hyphae from germinating hyphal fragments. The latter were floated onto onion agar in sterile distilled water, where germination took place within a few hours. Growth ensued quite rapidly and soon became difficult to follow because of the repeated branching of the hyphae.

The data from a single representative experiment run over a period of 52 hours are shown in Table III, and are represented graphically in Figure 3. No growth had occurred within this period at 9° C., but it was proceeding well at 12°. The growth maximum appeared to be around 18°, which is approximately 3° higher than in the case of germination of chlamydospores and hyphal fragments. Some growth occurred at 25°, while none could be observed after 52 hours at 28°. There is a notably larger amount of growth at 18° than at 15° or 24°. This curve ascends and descends more abruptly

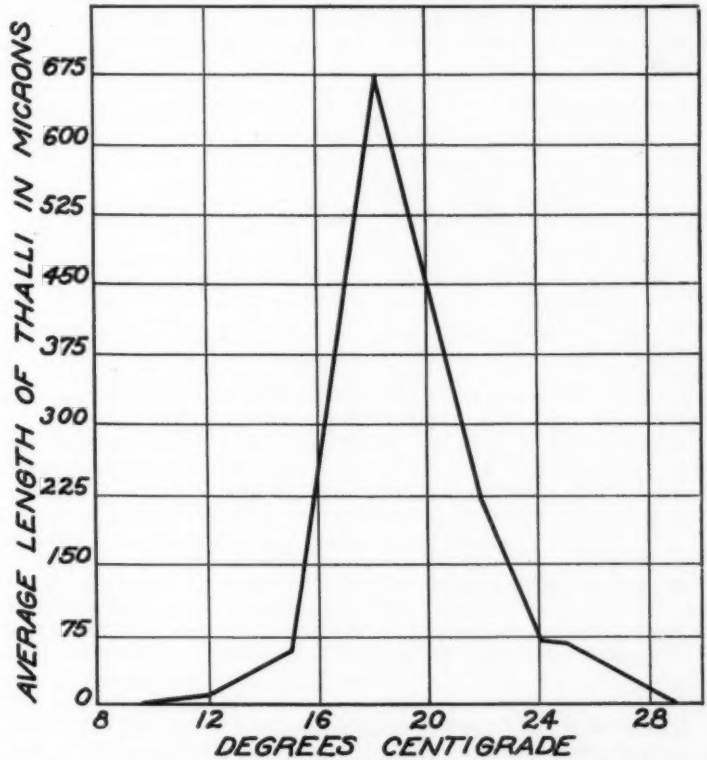


FIG. 3.—Relation of temperature to the growth of thalli from hyphal fragments. Data taken at 52 hours after beginning of the experiment. (See Table III)

between those points than do the germination curves. Apparently growth is not arrested as quickly by the higher temperatures as is germination, but at 24° and 25° the hyphae became noticeably weaker and more attenuated in appearance.

Following the 52-hour measurements the Petri dishes were returned to their respective incubators and growth was allowed to continue for some 15 days longer. By that time macroscopic colonies had developed at the favorable temperatures. Photographs after that period of five separate plantings of hyphal-fragment suspensions

on agar plates for each temperature are reproduced in Plate 1. Where no macroscopic growth resulted the point of inoculation is marked by a white circle. It is to be noted that no macroscopic growth had yet occurred at 9° C., while at 12° colonies were evident. It is apparent that growth is as abundant at 22° as at the earlier indicated optimum of 18°, and that the fungus grows very well over the range of 15° to 22°. At 25° there is evidence of retarded growth, while at 28° only one out of five plantings grew. A marked difference in the appearance of the colonies at optimum and at extreme temperatures was noticeable. At 22° the surface of colonies is prominently rugose, but somewhat less so at 15°, 18°, and 25°; at 12° and 28° the colonies have a raised velvety appearance, but no evidence of rugosity.

TABLE III.—*Relation of temperature to growth of thalli of Urocystis cepulae*

Average temperature	Average length of 25 thalli after 52 hours' incubation	Average temperature	Average length of 25 thalli after 52 hours' incubation	Average temperature	Average length of 25 thalli after 52 hours' incubation
° C.	*Microns*	*° C.*	*Microns*	*° C.*	*Microns*
3	0	15	86	25	67
6	0	18	675	28	0
9	0	22	214	32	0
12	11	24	74		

The question arose as to the viability of the hyphal fragments after exposure to near-maximum and near-minimum temperatures for this fortnight. It would be expected on *a priori* grounds that those at the low temperature would retain their germinative capacity, while at the higher temperatures the protoplast, as a result of increased metabolism, might expend its energy so rapidly as to have much-reduced vitality. Noble (*14*) showed for *Urocystis tritici* that spores germinating above 25° C. "spend themselves" much more rapidly than at lower temperatures. Plates which had been exposed to 3°, 6°, 9°, 28°, and 32° for the fortnight were therefore removed to room temperature (±20° C.). Examination 17 days later showed that normal colonies resulted on the plates held originally at 3°, 6°, and 9°, while on those held formerly at 28° and 32° no further growth occurred. It is evident, therefore, that temperatures of 28° or above not only prevent practically all growth on agar plates, but that hyphal fragments exposed to those conditions for two weeks in most cases die. On the other hand, prolonged exposure on agar plates to temperatures between 3° and 9°, though almost entirely preventing germination and growth, does not seem to be detrimental to the germinative capacity of hyphal fragments.

DISCUSSION OF EXPERIMENTAL RESULTS

The influence of external factors upon infection is expressed in the effect upon host and parasite separately or jointly. It also may or may not be cumulative. Previous studies (*21*) have shown that onion-smut infection occurs at as low a temperature as is ordinarily encountered by the host, and that abundant infection takes place

up to 25° C.; as one goes above 25° infection is rapidly lessened, until none occurs at 30°. The question at hand is: To what extent is this the result of direct inhibitive effects upon the fungus, and to what extent is it the result of host response?

Considering first the lower temperatures, we find that at abou 10° C. germination and growth of the fungus are very slow. Due to the reduced temperatures, we expect that metabolic activity is also much retarded, but we find that, aside from the retardation in activity, the hyphae are quite as able to cause infection as at slightly higher temperatures. Along with this slowing down of the fungus there is a corresponding retardation of growth of the host cotyledon (*21*), and in consequenec an extension of the susceptible period. Thus the coincident holding back of host and parasite due to low temperature only retards the progress of infection, but in the end does not appreciably reduce the amount of infection. As the temperature is increased up to 25° C. the rate of growth of both host cotyledon and parasite increases in somewhat the same proportion, and in consequence infection occurs as abundantly and the disease develops more rapidly.

Above 25° C. there is to be noted a rather abrupt reduction in germination and growth of the parasite. In contrast to the retarded growth at low temperatures, there is probably at these higher temperatures less vigorous development and a more rapid exhaustion of the organism, due to increased metabolism. In further contrast, there is not a corresponding decrease in top growth of the onion nor increase in the length of the susceptible period. In other words, the parasite is being retarded due to the unfavorable temperature, and it continues more or less rapidly to exhaust its food reserve, while on the other hand the host through only slightly reduced growth is advancing promptly to the condition of immunity. It will be seen, therefore, that in comparison with the condition at low temperatures where reduced host and parasite activity combine to permit extensive infection, the combination of circumstances at the high temperatures leads to just the opposite result. Whereas we may justly say that the inhibition of infection at 27° C. and above is in a large measure due to the direct effect of temperature upon the parasite, the fact must not be overlooked that at this higher temperature the host is stimulated to relatively rapid top growth, and consequently the susceptible period is not as prolonged as at the extremely low temperatures.

The direct effect of the various soil temperatures upon the host tissue in relation to infection should be studied further. The question is still open as to whether or not the seedlings grown at constant temperatures above 25° are sufficiently different in constitution to render them more resistant to the parasite than those grown at temperatures lower than 25°. If such is the case the direct influence of temperature upon the host tissue may be a factor in the limitation of infection. Another problem of interest in this connection concerns a study of the changes which the cotyledon and leaves undergo between the young stage, when they are susceptible to smut invasion, and the time of maturity, when they are immune.

CORRELATION WITH DISTRIBUTION IN AMERICA AND EUROPE

It may be well to review, in the light of our present knowledge, the history of the appearance and gradual spread of onion smut both in Europe and America. The first record in America is that by Ware (*22*) in 1869, at which time the disease was of sufficient economic importance to attract the serious attention of onion growers in the Connecticut River Valley. With the wide dissemination of spores within or upon bottom sets, the fungus has undoubtedly been introduced at one time or another into all parts of this country. As pointed out previously (*21*), following this general distribution it is now evident that the disease has become established and has assumed destructive proportions in most of our intensive onion-growing sections of the northern and central States, where the crop is spring-sown. On the other hand, the southern onion-growing sections, where autumn sowing is the general practice, have apparently remained free. This is the more noteworthy because of the intensity of onion culture in certain southern areas, and also because of the fact that the smut is frequently and widely introduced into the South on northern-grown onion sets.

Although it has been generally assumed that onion smut was of American origin and was introduced thence into Europe, it is well to note that the first recorded collection of the fungus was made on *R. ascalonicum* in Denmark (*12, p. 273*) in 1864. During the two decades which followed this it was reported more widely in Europe than in America.[3] Passerini described a form from Italy on *Allium magicum* in 1875 (*19, p. 204–205*).[4] Hallier (*9*) reported *Urocystis cepulae* on onion from Germany in 1877. Schroeter (*18, p. 378–379*) collected it in southern France in the same year. Cornu (*4*) found it near Paris in 1879; Frank (*8*) near Leipzic, Germany, in 1880; and Malbranche (*13*) at Rouen, France, in 1881. So by 1880 it was widely distributed in western Europe, while in America it had not been reported outside of New England.

It is of interest, therefore, to compare 40 years later the regional distribution and destructiveness of the two diseases on the two continents. The senior writer had an opportunity to visit, with this point in mind, a number of the intensive onion-growing sections of western Europe in 1922. The onion crop north of central France is sown chiefly in the early spring, but to some extent in the autumn. In either case, however, there is probably at no time during the sowing or growing seasons a prevalent soil temperature which is unfavorable to onion-smut spore germination and growth. On the contrary, it would seem that conditions more nearly approach the ideal for its development than in any other location. As one goes southward,

[3] There was some tendency in the beginning to interpret the Urocystis on Allium as a form of *Urocystis colchici*, but this was early discredited by Farlow (*7, p. 114*).

[4] Passerini described *Urocystis magica* on *Allium magicum* (synonymous with *Allium nigrum*) collected at Parma, Italy, and distributed in it Thümens's Mycotheca Universalis, No. 223. Farlow (*7, p. 114*) compared this collection with *Urocystis cepulae* and decided that they were identical. The present writers have compared with *Urocystis cepulae* two collections made in northern Italy on *Allium magicum* (*nigrum*) and distributed by Kühn in Rabenhorst's, Nos. 2585 and 2586, as *Urocystis colchici* var. *allii-nigri*, and are in agreement with Farlow that the latter is distinct from *U. colchici* and probably identical with *Urocystis cepulae*. This is further supported by the experiments of Anderson (*2*), in which it is shown that *Allium nigrum* is susceptible to the onion-smut organism. Anderson, however, does not believe the forms on *Allium magicum* (*nigrum*) and on *Allium cepa* to be identical. Although the relation of the onion form to those on wild species of Allium is still unsettled, there is little doubt that the onion form was of wide occurrence in eastern Europe before 1880.

however, there is a longer, drier, and warmer summer, which is more unfavorable for the establishment of the fungus accidentally introduced into the soil. There is along with this a general practice of sowing the onion seed toward the close of the warm summer, thus bringing on the young seedling during its period of susceptibility to smut in a warm soil and undoubtedly oftentimes at a temperature which would completely check the parasite. Although it must be true that onion-smut infection could occur in southern Europe if the plant were exposed under the proper conditions, it seems significant that in Europe, as in America, as one goes southward the combination of environmental conditions and cultural practices becomes the more unfavorable for the establishment and spread of the organism.

Citing now the actual findings, it was noted that onion smut is now common and destructive at Edinburgh, Scotland, and in Northumberland (*5*), the most northerly county of England. Although the disease is now restricted by local quarantine in England, it has been found to assume destructive proportions where introduced in Northampton (*5*), a short distance north of London. In Denmark it has been reported occasionally, but the minor importance of the onion crop in that country would prevent its ever being more than of incidental occurrence. In the intensive onion-growing section of Germany, near Calbe, the disease is very destructive (*23*). In Holland it occurs in the onion-growing region of Zealand. Though it is apparently being checked by judicious crop rotation, the writers' observation of a single field where continuous cropping of onions had occurred for several years left no doubt that conditions were very favorable for the multiplication of the parasite. The disease is common in northern France. In southern France it is apparently little known among onion growers. It had not been found in the onion-seed-growing section at St. Remy, although the government inspector had searched repeatedly for it. In Spain the only authentic report of the disease, according to Dr. Gonzalez-Fragoso, of Madrid, is a single collection at Seville. It has not been found at Valencia, which is the largest and most intensive onion-producing section of Europe, or at La Coruna, a smaller but very intensive section in the northwest of Spain. Personal survey of these localities by the senior writer failed to reveal the disease. In Italy no authentic report of the disease was found other than the early collections of Passerini (*19, p. 204–205*) and Kühn on *Allium magicum*, already cited.

It would appear, therefore, that although onion smut was present in Europe from Italy to Denmark four decades ago, the disease has multiplied to the point of economic significance only in the more northern, cooler areas. Lack of dissemination alone could hardly account for this restriction. Moreover, the almost exact coincidence of this condition with that in America, where we know that onion smut is being widely disseminated annually on bottom sets, leads us to believe that climatic differences have an important bearing. The longer period of high temperatures in the upper layers of soil probably restricts the establishment of the fungus, while in many cases the temperature immediately after seed is sown is sufficient to check or completely inhibit infection.

SUMMARY

The soil environment, and especially the soil temperature during the short period following germination when the onion seedling is susceptible to infection by the smut fungus, *Urocystis cepulae* Frost, has an important bearing on the amount of disease which results. Previous studies have shown that abundant infection occurs at temperature as low as 10° to 12° C., which is nearly as low as germination and growth of onion ordinarily occurs. There appears thus to be no low point at which the host escapes the disease. Infection occurs equally well up to 25°, but above that a rapid reduction occurs, while at 29° or above the onion seedling will grow free from the disease.

The purpose of the present study has been to determine whether this temperature inhibition is the result of direct effect of the temperature upon the parasite, of host response to temperature, or the combined effect of both.

By studying the germination and growth of the fungus on artificial media the effect of temperature on the parasite under these conditions was determined. It was found that the minimum temperature for germination and growth of *Urocystis cepulae* was very close to that of the onion plant. The optima for chlamydospore germination, hyphal-fragment germination, and vegetative growth of the thallus lie between 13° and 22° C. Above 25° there is a decided reduction in the amount of germination which occurs, and the growth of the hyphae becomes more meager as the temperature rises. Such hyphae as do appear above 28° not only grow very slowly, but soon lose their viability upon continued exposure to these higher temperatures. On the contrary, protracted exposure to near-minimum temperatures does not so affect the slowly growing thallus.

There is no appreciable change in the rate of growth of the host or in the length of its susceptibility when grown slightly above or below the critical point (29° C.) above which no infection occurs. Further study is needed to determine whether there are changes in the host tissue at the high temperatures which render it immune to infection. The marked inhibitive effects of temperatures above 25° upon the parasite, with the maximum lying only slightly above 29°, show the direct influence of temperature upon the parasite to be a primary factor in the limitation of infection.

Although the smut organism is being continually introduced throughout the country on northern-grown onion sets, the disease has as yet never been recorded in the intensive southern onion-growing regions, while it has become established in most of the intensive northern sections. Likewise, in Europe the disease is largely confined to the northern, cooler areas. The results of this study strengthen the theory advanced earlier that temperature is one of the important factors which bring about this regional limitation of the disease.

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EFFECTS OF WEATHER ON THE WORLD DISTRIBUTION AND PREVALENCE OF CITRUS CANKER AND CITRUS SCAB[1]

By GEORGE L. PELTIER, formerly *Plant Pathologist, Alabama Agricultural Experiment Station, and Agent, Bureau of Plant Industry, United States Department of Agriculture,* and WILLIAM J. FREDERICH, formerly *Assistant Pathologist, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Basing the greater part of this paper on the behavior of citrus scab and canker as determined under natural and controlled conditions in Alabama (*4, 5*),[2] an attempt will be made to correlate in a general way the effects of weather on the world distribution of these two diseases. In so doing specific examples will be presented and compared. By such a study the writers hope to show the types of climate that are favorable or unfavorable for the development and spread of these diseases, the weather conditions essential for or militating against their successful propagation, and the type of climate which favors the development of one disease and inhibits the other, in the more important citrus-growing regions of the world.

HISTORY AND DISTRIBUTION

In all probability, citrus scab (*Cladosporium citri* Massee) is indigenous to southeastern Asia, the native home of Citrus. From South China it was probably introduced into Japan and carried from there, directly or indirectly, to the citrus districts, as indicated in a previous publication (*5*).

Citrus canker (*Pseudomonas citri* Hasse) appears to be of more recent origin than scab. The first account of the disease dates back to the introduction of the navel orange into Kyushu Island, Japan, about 1899. For a further account of the early history of citrus canker in Japan, consult paper by Tanaka (*8*). With the exception of the Far East, every report of canker can be traced back either directly or indirectly to Japan. While canker had been found by both Lee (*3*) and Reinking (*7*) along the coast of South China, indications are that it is not present in the interior, showing that it probably was introduced into the citrus-growing region of the coast of South China.

[1] Received for publication May 6, 1925; issued February, 1926. Published with the approval of the director of the Alabama Agricultural Experiment Station. The paper is based on cooperative investigations between the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Plant Pathology, Alabama Agricultural Experiment Station. Presented before the Botanical Society of America, Toronto, Canada, December, 1921. Since that time several papers dealing with certain phases of citrus scab have appeared, to wit:

JENKINS, A. E. THE CITRUS SCAB FUNGUS. Phytopathology 15: 99–104, illus. 1925.

TANAKA, T. A BRIEF HISTORY OF CITRUS SCAB IN JAPAN. Phytopathology 13: 492–495. 1923.

WINSTON, J. R. CITRUS SCAB: ITS CAUSE AND CONTROL. U. S. Dept. Agr. Bul. 1118, 38 p., illus. 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 164.

The distribution of these diseases is shown roughly in Figure 1. In Japan, Formosa, South China, Hawaiian Islands, the Gulf coast of the United States,[3] and South Africa both diseases are present. In the Philippine Islands, North Territory of Australia, Borneo, Java, Siam, Indo-China, and Singapore only canker has been found; while in New South Wales and Victoria in Australia, New Zealand, Ceylon, the West Indies, and Mexico only scab has been reported. Neither disease has ever been reported from California or the Mediterranean countries.

REVIEW OF PREVIOUS WORK

The primary object of this paper is to point out the type of weather which favors or inhibits canker and scab in the more important citrus districts of the world. At the same time, an opportunity is afforded of comparing two diseases attacking the same plant parts but caused by different types of organisms. A brief review of the outstanding characteristics of these two diseases, together with the cardinal points for the growth, of the pathogenes in culture, of the host plants, and for infection and development of the diseases under controlled and field conditions, will suffice at this point (Table I).

[3] Owing to the success of the eradication campaign in the Gulf Coast States, few active citrus canker areas are present at this time.

TABLE I.—*Summary of the chief characteristics and cardinal points for the growth of the pathogenes in culture, and on the infection and development of the diseases under controlled conditions*

Citrus scab	Citrus canker
CAUSE	
A parasitic fungus, *Cladosporium citri* Massee.	A parasitic bacterium, *Pseudomonas citri* Hasse.
HOST PLANTS	
With the exception of *Poncirus trifoliata*, strictly limited to some of the citrus fruits and their hybrids. Differences in susceptibility and resistance not well marked. Apparent resistance in some cases question of disease escape.	Attacks wide range of Rutaceous plants, including many wild relatives, all citrus fruits and hybrids so far tested. Marked differences in the susceptibility and resistance of these different plants apparent.
AGE AND PART OF PLANT ATTACKED	
Unfolding leaves, occasionally young and tender twigs, and small, young fruits. Infection limited to young growth. Nursery stock more susceptible than grove trees.	Usually full-grown, nonmature leaves, occasionally young and old leaves, angular wood, round wood of some few varieties of Citrus, and young fruits. Infection possible throughout the growing season. Nursery stock more susceptible than grove trees.
DESCRIPTION OF THE DISEASE	
Scab may appear on either side of the unfolding leaves as small, glistening spots. Later these spots enlarge, become yellowish in color, and are always raised on one side of the leaf and depressed on the other, producing a wartlike projection. They become darker in color with age and more misshapen, in many cases distorting the leaves. Little or no defoliation occurs.	Spots usually appear on the under side of the leaves, as small, yellow, translucent, oily pimples. Later these rupture to form round, brown, raised, corky spots, with an oily outline and sometimes a wide yellow margin. The spots break through to the upper surface to form a raised, corky spot similar to that produced on the lower side. No malformation of the leaves is produced. More or less defoliation ensues.

TABLE I.—*Summary of the chief characteristics and cardinal points for the growth of the pathogenes in culture, and on the infection and development of the diseases under controlled conditions*—Continued

DESCRIPTION OF THE DISEASE—Continued

Citrus scab	Citrus canker
Twig infection occurs occasionally. The spots are like those on the leaves, except they are somewhat flatter.	Young spots on angular wood similar to those on leaves. As they become older, they become more corky and have a tendency to girdle and eventually kill the twigs. Spots on the round wood are like those on the angular wood and are usually produced while the twig is triangular. Round-wood infection occurs only rarely and then only on grapefruit and allied plants. In all cases the cankers are superficial, rarely penetrating beyond the cambium layer.
Fruits are attacked when small and become warty and misshapen.	On young fruits spots like those discussed above occur. They are more or less superficial and corky.
Primarily a foliage and fruit disease. Production of cull or unmarketable fruits most serious aspect of the disease.	Primarily a foliage and twig disease which defoliates and kills the young twigs, consequently lowering the vitality of the trees. The disease may also cause a spotting and dropping of the fruits.

TEMPERATURE RELATIONS OF THE ORGANISM IN PURE CULTURE

Minimum, 56° F. (or below); optimum, 69.8° F.; maximum, 89.6° F. Spore production limited to temperatures between 60.8° and 81.5° F.	Minimum, 42° F.; optimum, 86° F.; maximum, 95° F. For periods of 24 hours or longer. Thermal death point 120° to 129.6° F.

TEMPERATURES ESSENTIAL FOR INFECTION AND DEVELOPMENT OF DISEASE

Minimum, 59° F.; optimum, 68° to 69.8° F.; maximum, 74.3° F.	Minimum, 68° F.; optimum, 86° F.; maximum, 95° F. (with exceptions).

METHOD OF OVERWINTERING

By means of mycelium and spores in old spots on leaves or lodged in bud scales of current year's growth.	In old spots, on leaves, angular wood and round wood of current season's growth. By means of latent infection.

MODE OF ENTRANCE

Direct penetration of the epidermis and possibly wounds.	Stomata, lenticels, and wounds.

PREREQUISITE FOR SUCCESSFUL INFECTION

Temperatures between 59° and 74.3° F.; optimum at 68° to 69.8° F. Free moisture on surface of leaves for several hours or more. Young unfolding leaves, tender twigs, and young fruits.	Temperatures between 68° and 95° F.; optimum at 86° F. Free moisture on surface of leaves for 20 minutes or more. Rapidly growing leaves, angular wood, and young fruits throughout the growing season.

PERIOD FOR INITIAL INFECTION

Long; several hours or more.	Short; 20 minutes or more.

PERIOD OF INCUBATION

Six days or more; under optimum controlled conditions, 6 days to 2 weeks.	Forty-eight hours or more; under optimum controlled conditions, 4 days or less.

CONTROL

Spraying.	Eradication (United States, South Africa, Australia).

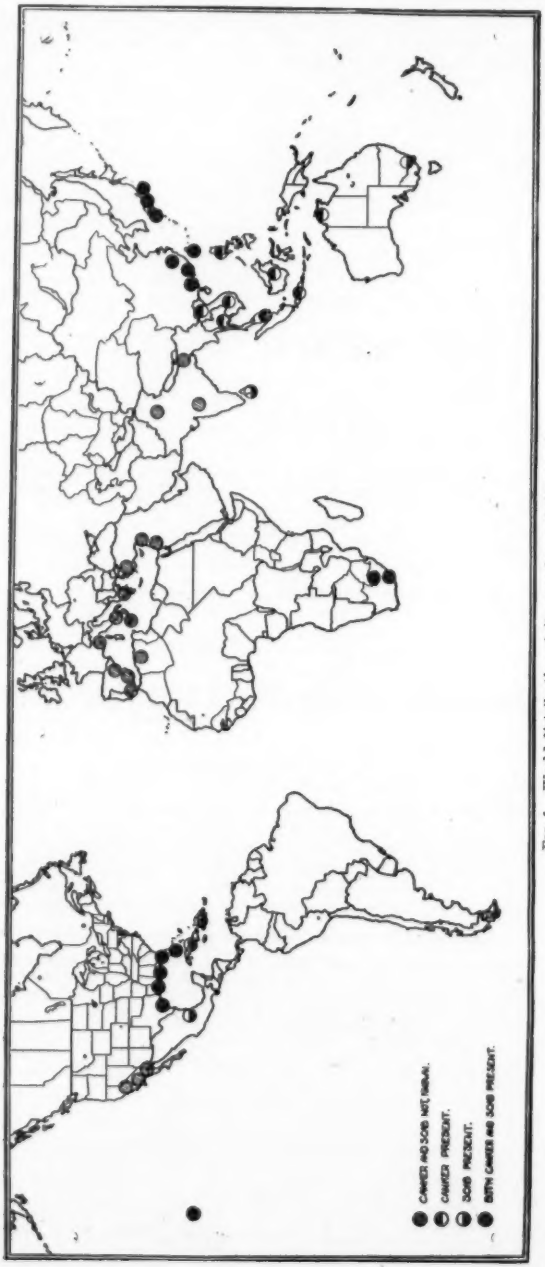

FIG. 1.—World distribution of citrus canker and citrus scab

The senior writer has established, under controlled conditions, the cardinal points for growth of a few types of citrus plants. Using the weather records obtained at Mobile, Ala., and four years' observations of plants of different types in the field, the writers were able, in a general way, to establish the cardinal points for growth of the more important citrus types under southern Alabama conditions. Likewise, under field conditions, they were able to establish the cardinal points for infection and development of canker and scab from their observations and the use of weather records. They found that the same cardinal points for infection and development of these diseases, as determined at constant temperatures under controlled conditions, are, for the most part, equivalent to weekly or monthly mean temperatures encountered under field conditions. For a detailed discussion of the various points summarized and those not considered here, the readers are referred to previous publications (*4*, *5*) of the writers.

In presenting the data which follow the writers have tried to be as clear and concise as possible. The greatest difficulty arises from the fact that they are attempting to discuss the effect of weather on two distinct diseases which vary considerably in their reaction, not only to environmental conditions but to the host plants themselves. To avoid complexity, a discussion of the relation of the host plants to environmental influences has been omitted.

In Table II are listed the normal mean monthly temperatures, precipitation, and number of rainy days where these have been available for a period of years. Throughout the discussion of the influence of weather on these diseases the normals have been used.

TABLE II.—*Climatic data for various points in the citrus regions of the world* [a]

A.—MONTHLY MEAN TEMPERATURES (FAHRENHEIT)

Station	Number of years	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Miami, Fla.	17	67.3	68.8	72.0	74.2	78.6	80.4	81.9	82.0	81.5	77.8	72.0	68.0	75.4
Tampa, Fla.	29	60.1	62.3	66.3	71.0	76.4	80.0	81.2	81.4	79.7	73.8	66.8	61.2	71.7
Jacksonville, Fla.	48	53.9	56.9	61.9	67.6	74.2	79.0	80.9	80.1	77.3	69.6	61.3	55.2	68.2
Mobile, Ala.	47	49.8	53.2	59.1	66.0	75.6	79.1	80.5	79.7	76.5	61.1	57.5	51.5	66.1
Hongkong, China	15	59.6	59.8	64.3	73.0	79.6	83.5	84.5	83.8	82.3	78.3	70.2	62.7	73.5
Swatow, China	------	57.0	57.0	64.0	71.0	75.0	82.0	84.0	84.0	81.0	75.0	70.0	60.0	71.7
Foochow, China	2	50.6	48.8	58.0	62.7	67.5	76.0	80.4	80.8	74.7	68.8	62.1	52.4	65.2
Nagasaki, Japan	25	42.4	42.4	48.7	57.9	64.4	71.1	78.3	80.0	74.5	64.6	54.9	46.0	60.4
Kagoshima, Japan	20	44.6	44.6	51.4	59.7	65.1	71.2	77.9	79.3	75.0	66.0	57.0	48.4	61.7
Osaka, Japan	25	39.0	39.4	45.3	56.1	63.7	71.8	78.2	81.3	74.1	62.2	51.8	43.0	58.8
Nagoya, Japan	15	37.9	38.7	45.3	55.9	63.5	70.9	77.9	79.9	73.6	61.7	51.1	41.7	58.2
Habana, Cuba	20	70.3	72.0	73.4	76.3	79.2	81.3	81.9	81.5	80.4	77.9	74.7	71.6	76.7
Manila, P. I.	20	77.0	77.2	80.2	82.9	83.5	82.2	80.8	80.8	80.6	80.4	79.0	77.4	80.2
Darwin, Australia	37	84.0	83.5	84.2	84.2	81.8	77.3	77.2	79.4	83.0	85.2	85.7	85.3	82.6
Pretoria, South Africa	7	70.5	68.5	65.7	62.1	57.4	51.8	51.8	55.0	61.7	65.7	66.9	67.8	62.1
Catonia, Italy	(c)	51.4	52.5	56.3	60.3	66.2	74.1	79.5	79.3	75.9	68.9	60.1	54.0	64.9
Palermo, Italy	(c)	50.5	52.2	54.7	58.6	64.0	70.7	76.3	76.6	73.4	67.3	59.4	53.4	63.1
Seville, Spain	10	52.0	55.9	59.5	63.9	69.6	78.1	84.7	84.9	78.1	68.4	60.1	52.9	67.3
Valencia, Spain	10	48.6	51.4	53.4	57.4	62.8	69.4	74.8	74.5	69.8	63.1	56.8	50.5	61.0
Los Angeles, Calif.	42	54.3	55.1	56.9	59.4	62.5	66.7	70.4	69.7	69.5	64.6	60.3	56.5	62.2
San Diego, Calif.	48	54.0	54.6	56.2	58.2	60.8	63.8	66.9	68.7	66.9	63.0	59.0	55.7	60.6
Riverside, Calif.	37	51.1	52.7	60.1	60.4	65.2	70.6	76.3	76.4	72.1	64.2	58.2	53.4	63.4
Fresno, Calif.	32	45.4	49.2	54.9	61.2	68.4	75.8	77.8	81.2	74.3	64.7	54.6	46.8	62.9
Fort McIntosh, Tex.	44	55.5	60.4	68.6	75.6	81.2	85.5	87.2	87.0	82.4	73.7	63.6	56.4	73.1
Do.	[b] 1916	60.6	61.0	70.9	73.0	82.3	89.0	85.0	82.8	81.4	72.6	61.8	55.1	73.0

[a] Climatic data supplied by J. Warren Smith, agricultural meteorologist, Weather Bureau, U. S. Department of Agriculture.
[c] Long record, but exact years not given.
[b] For the year 1916 only.

TABLE II.—*Climatic data for various points in the citrus regions of the world*—Continued

B.—MONTHLY MEAN PRECIPITATION (IN INCHES)

Station	Number of years	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Miami, Fla	28	3.45	2.70	2.72	2.59	6.37	7.89	7.24	7.60	9.61	10.54	2.55	2.24	65.50
Tampa, Fla	29	2.80	3.27	2.81	1.85	2.92	8.34	8.43	8.59	7.41	2.97	1.72	2.02	53.13
Jacksonville, Fla	48	3.12	3.43	3.52	2.72	4.25	5.53	6.20	6.21	8.03	5.06	2.19	2.99	53.25
Mobile, Ala	47	4.85	5.36	7.17	4.35	4.00	5.95	7.04	6.81	5.02	3.18	3.74	4.57	62.04
Shamsni, South China [b]	10	1.49	2.47	5.89	8.10	9.40	9.00	9.32	8.60	7.57	4.58	1.33	1.81	69.56
Swatoe, South China	10	1.44	1.71	3.76	7.86	8.12	9.77	6.92	6.60	6.24	3.13	1.81	2.27	59.63
Foochow, South China	10	1.75	4.14	5.26	4.39	5.57	8.10	6.66	7.47	9.22	2.77	2.09	2.20	59.52
Nagasaki, Japan	25	2.96	3.03	5.04	8.12	7.46	11.10	10.16	7.73	7.09	5.52	3.58	3.64	75.41
Kagoshima, Japan	25	3.45	3.31	6.11	9.11	9.65	10.00	11.20	7.44	8.71	5.13	3.74	3.53	81.38
Osaka, Japan	25	1.90	1.96	4.22	6.19	5.20	7.23	6.86	4.17	6.77	5.01	3.14	1.89	54.54
Nagoya, Japan	15	2.12	2.16	5.48	6.69	6.58	9.11	8.88	6.76	10.40	5.91	3.62	2.24	69.95
Habana, Cuba	26	2.72	2.28	1.81	2.83	4.49	7.17	5.04	6.02	6.69	7.40	3.07	2.17	51.69
Manila, P. I.	42	1.10	.39	.67	1.18	4.06	9.80	15.32	13.78	14.53	7.48	5.16	2.28	75.75
Alice Springs, Australia	39	1.68	1.73	1.24	.90	.60	.57	.46	.40	.41	.72	.90	1.32	10.93
Pretoria, South Africa	18	5.55	3.94	3.46	1.06	.59	.20	.16	.16	1.10	1.81	3.74	4.21	25.98
Catonia, Italy	[c]	3.27	2.24	2.01	1.34	.67	.28	.08	.35	1.22	2.60	3.54	3.38	20.98
Palermo, Italy	[c]	4.09	3.07	3.19	2.64	1.34	.59	.31	.55	1.50	3.98	3.98	4.53	29.77
Seville, Spain	30	2.13	1.85	2.48	1.85	1.69	.55	.04	.16	.75	1.93	2.40	2.72	18.55
Valencia, Spain	30	1.34	1.18	1.54	1.54	1.69	.83	.47	.35	2.99	3.13	1.97	1.89	19.10
Los Angeles, Calif	42	2.84	2.91	3.00	1.13	.48	.07	.00	.00	.06	.77	1.48	2.90	15.64
San Diego, Calif	69	2.00	1.96	1.70	.74	.41	.03	T.	.00	.06	.46	.83	1.82	10.01
Riverside, Calif	37	2.01	1.98	2.34	.68	.35	.05	.02	.14	.14	.54	.77	1.57	10.59
Fresno, Calif	32	1.60	1.33	1.76	0.71	.63	.10	T.	.00	.27	.72	1.03	1.53	9.68
Laredo, Tex	[d] 1916	1.63	.00	.00	.34	.07	.15	3.80	4.74	3.71	3.58	.35	.00	18.37
Fort McIntosh, Tex	44	.75	1.00	1.00	1.73	2.29	2.28	1.77	2.52	2.69	1.38	1.08	1.08	19.57

C.—NUMBER OF RAINY DAYS (0.01 INCH OR MORE)

Miami, Fla	13	4	3	4	4	6	9	8	9	11	9	4	3	74
Tampa, Fla	19	8	8	6	5	7	15	18	19	16	7	5	7	121
Jacksonville, Fla	37	9	9	8	7	10	13	15	15	14	10	8	8	126
Mobile, Ala	38	11	10	10	8	8	12	15	14	10	6	7	7	121
Shamsni, South China [b]	8	9	12	17	18	18	16	15	16	13	7	5	8	154
Swatow, South China	8	7	10	14	13	14	16	14	11	10	5	6	8	128
Foochow, South China	8	9	13	16	18	15	13	10	12	11	11	8	8	144
Nagasaki, Japan [d]	25	16	13	14	15	14	16	14	13	13	11	12	16	167
Kagoshima, Japan [d]	20	13	12	16	14	15	17	17	13	14	10	9	12	162
Osaka, Japan [d]	20	9	9	13	14	13	14	13	11	13	11	9	9	138
Nagoya, Japan [d]	15	10	8	13	13	13	14	16	12	14	11	10	9	143
Manila, P. I. [d]	36	5	3	3	4	9	16	21	21	20	16	12	8	138
Los Angeles, Calif	42	7	6	7	4	2	1	0	0	1	3	3	6	39
Fort McIntosh, Tex	44	2	2	3	3	4	4	3	3	4	2	4	2	36
Laredo, Tex	[d] 1916	6	0	0	4	2	2	8	4	5	3	1	0	35

[b] Near Canton.
[c] Long record, but exact record not given.
[d] 1 mm. or more constitutes a rainy day.
[d] For the year 1916 only.

Except in a few instances, the writers have not attempted a detailed analysis of the weather in all the citrus-growing regions. Their sole idea is to show, in a general way, the effects of weather on development and spread of scab and canker in the more important citrus-growing regions of the world. The detailed analysis of the influence of weather prevailing in different years on the development or inhibition of one or the other diseases in the localities mentioned, must be left to the citrus pathologists of the respective countries.

On looking over the graphs to be discussed forthwith, it will be noted that the minimum (59° F.) and maximum (75° F.) temperatures for scab infection, and also the approximate time limits in which it can occur, are indicated. Likewise, the minimum temperature (68° F.) and time limits for canker infection are indicated. The minimum temperature (59° F.) for scab infection also happens

to be the minimum temperature for the growth of grapefruit plants, while the minimum temperature (68° F.) for canker infection is almost the optimum temperature (69.8° F.) for scab infection.

TYPES OF WEATHER FAVORABLE TO THE DEVELOPMENT OF BOTH SCAB AND CANKER

Both canker and scab are prevalent in the Gulf Coast States of America, South China, Japan, South Africa, Formosa, and the Hawaiian Islands (fig. 1). Citrus growing is an important industry in only the Gulf States, South China, and Japan, so at this point the relation of weather to scab and canker will be discussed and compared for these three localities only.

In Florida the two commercially important citrus fruits are the sweet orange and grapefruit. Both are susceptible to canker. The sweet orange, with few exceptions, is nonsusceptible to scab. In northern Florida the mandarin orange is grown somewhat extensively. These oranges are rather resistant to canker, but are susceptible to scab. In southern Alabama the Satsuma orange is the most important type grown commercially; grapefruit and sweet oranges are also grown in a limited way. Similarly in the Gulf Coast States to the west of Alabama, about the same type of citrus trees are grown, except in Louisiana, where more sweet oranges are grown.

In South China the predominating citrus fruits are the coolie orange, oranges of the mandarin type, and pummelos. So far as known no reports of scab on the coolie orange have been recorded, although Reinking (*6*) reports scab on *Citrus sinensis*. Both scab and canker have been reported by Reinking (*7*) on two other citrus types, as well as on lemons.

In Japan the mandarin oranges predominate, followed by pummelos and navel oranges. Here again no reports of scab on varieties of *Citrus sinensis* Osbeck have ever been made, so far as the writers know.

From a commercial standpoint, the effects of scab on grapefruit is the most serious aspect of the disease in Florida, although scab attacks lemons, sour oranges, and Satsuma and other mandarin oranges.

It will be seen from Figure 2 that at Miami, Fla., the optimum temperature (68° to 69.8° F.) for scab infection is at hand just at the time the first growth of grapefruit is unfolding. At Tampa, grapefruit can make a start before the optimum temperatures for scab infection are present, and so may escape infection in some seasons. Both at Mobile, Ala., and Jacksonville, Fla., during normal years, grapefruit completes its first growth before optimum temperatures for scab infection are at hand. A detailed discussion of the question of scab escape, and the behavior of the host plants in relation to scab infection, has been presented in a previous publication (*5*).

By the end of May mean temperatures at all points are above the maximum (75° F.) for scab infection. It should be borne in mind that the prevailing temperatures do not interfere with the subsequent development of scab, once infection occurs.

As far as temperature is concerned, then, infection may be expected to take place any time after new growth starts in the spring until the end of May, along the Gulf coast. However, in each locality, growth of the various citrus plants starts out at different dates, owing to differences in the internal and external factors which influence active growth after dormancy. With some exceptions, the maturity of the first growth, and the presence of sufficient moisture at the time optimum temperatures for scab infection are at hand, determines to a large extent the amount of scab.

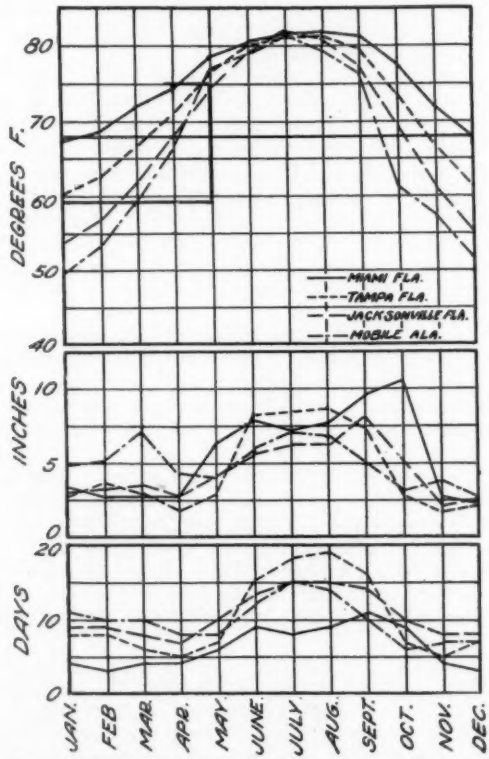

FIG. 2.—Climatic data for four points in the Gulf Coast States, and their relation to citrus canker and citrus scab

During the interval between the starting of the citrus plants and the end of May there are, as a rule, enough rainy days to meet the second requisite for successful scab infection in Alabama.

As has been pointed out in a previous publication (*5*), in Alabama a mean temperature below the normal (59.1° F.) for the month of March can be used as an indication of a scab epidemic, while a mean temperature above normal is indicative of a light scab season.

Fawcett (*2*) has pointed out that during the season of 1915, which was a very bad scab year in Florida, the mean temperature (62.6° F.)

for February, March, and April at Tampa was the lowest recorded over a 10-year period. So there, as in Alabama, low mean temperatures during the development of the first spring growth are conducive to a scab epidemic, especially when sufficient moisture for maximum infection by the spores of the fungus is at hand. On the other hand, temperatures above normal for this period result in the rapid maturity of the plants and so favors such scab escape. It should be pointed out in this connection that, especially in south Florida, the lack of rain during the development of the first growth may be a limiting factor in the prevalence of scab. At Miami the normal number of rainy days for February, March, and April is only 11, while at Tampa it is 19, and at Mobile for a corresponding period (March, April, and

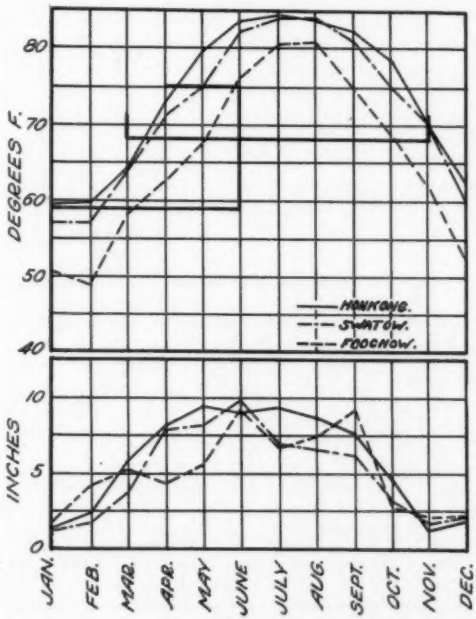

FIG. 3.—Climatic data for three points in South China, and their relation to citrus canker and citrus scab

May) there are 28 rainy days. Thus the rare occurrence of low mean temperatures and sufficient moisture during the spring months would favor the absence of frequent scab epidemics in southern Florida.

However, some scab would be present in this region during each season, as normally the optimum temperatures (68.0° to 69.8° F.) for scab infection are at hand when grapefruit starts in the spring. The presence of sufficient moisture would naturally increase the amount of scab. Fawcett's (*1*) statement that "it was of some interest to note that the scab appeared to increase in its injury to grapefruit as one passed south in Florida and appeared to become even more severe and common in Cuba and the Isle of Pines" is substantiated.

In South China the weather conditions essential for scab infection are very much similar to those in the Gulf coast regions, with one exception; namely, that maximum temperatures (75.0° F.) for infection are not reached until somewhat later, even though temperatures suitable for growth of the host plants are reached at about the same time (fig. 3). The more generous precipitation which occurs at all points in South China during the development of the first spring

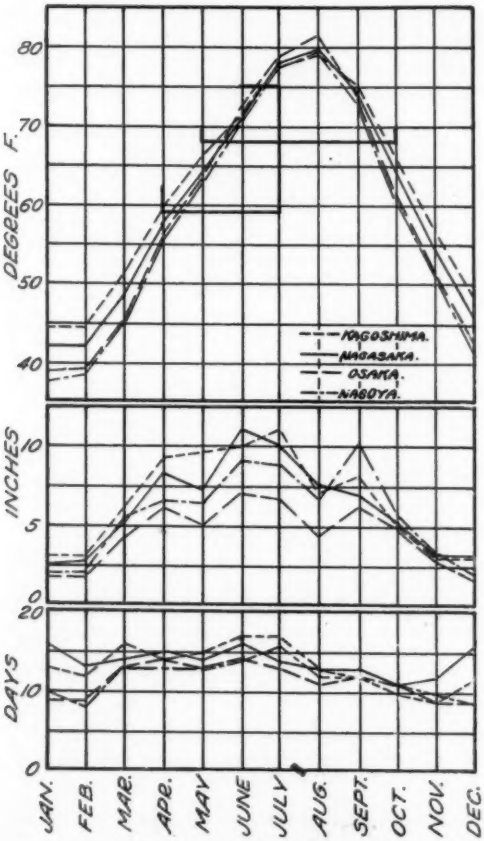

FIG. 4.—Climatic data for four points in Japan, and their relation to citrus canker and citrus scab

growth, favors more severe scab epidemics. Thus, we should expect scab to be more severe in South China than in the Gulf Coast region of the United States.

Likewise, the weather conditions in Japan (fig. 4) are much more conducive to scab than in the Gulf Coast States. Suitable temperature and sufficient precipitation for scab infection to occur prevail in Japan until into July. Judging from reports and statements concerning the prevalence of scab in Japan, it is considered a much more

serious disease than in our Gulf Coast States where we expect an epidemic only during certain years.

In the Gulf Coast States weather conditions essential for the development and spread of canker prevail, in most cases, throughout the growing season (fig. 2). The length of the growing season varies considerably for points like Miami and Jacksonville, Fla. The writers found that canker was more and more severe as they went further southward in Florida during the early days of the eradication campaign. During the summer months when mean temperatures above 80.0° F. prevail, together with plenty of precipitation, optimum conditions for the development and spread of canker are at hand. High mean temperatures and plentiful precipitation also stimulate a rapid and rank growth of the citrus trees, making them more susceptible to canker.

At Hongkong and Swatow in South China (fig. 3), still higher mean temperatures and more precipitation prevails during the summer months. Thus, canker is as serious in these localities as in the Gulf Coast States, if not more so. At Foochow canker would be active from May through October, with optimum conditions prevailing in only July and August.

The temperature curves for points in the Gulf Coast States (fig. 2) and South China (fig. 3) are rounding curves, while the ones presented for localities in Japan (fig. 4) run up to a peak with a maximum at about 80.0° F. in August. In other words, optimum temperatures for canker would prevail only during August. However, at all points there is a decided drop in the amount of precipitation during August, which would to some extent offset the influence of the higher mean temperature in the development and spread of canker. Statements and reports from Japan indicate that canker is not as serious a disease as in the Gulf Coast States.

The West Indies have been extremely fortunate in not having citrus canker brought into the islands. However, should canker be introduced at any time, it would be a very serious disease. One has but to compare the weather conditions at Habana, Cuba, with those of localities where canker is a serious disease to appreciate this statement.

Citrus scab is a rather serious disease, especially on grapefruit, in Cuba, Porto Rico, and the Isle of Pines. The maximum temperatures (75° F.) for scab infection are reached by the end of April at Habana, so any infection of grapefruit must necessarily occur before that time. However, compared with Miami, the temperature and precipitation curves for Habana and Miami are very similar (fig. 2).

As at Miami, optimum temperatures for scab infection are at hand when the first growth occurs, so the limiting factor would be sufficient moisture. Scab would be expected to be more severe during a season of frequent rainfall than in seasons when rainfall was light and infrequent.

In South Africa indications are that a thriving citrus industry is being built up. Navel oranges, grapefruit, and mandarin oranges are grown there. As was the case in Japan, citrus canker was not recognized as a distinct disease until susceptible grapefruit plants were introduced from Florida. It is now known that canker was brought into South Africa directly from Japan on nursery stock (*Poncirus trifoliata*).

At Pretoria, South Africa, temperatures are ideal for the development of scab (fig. 5), but here again we have both a dry season during the winter months and a small amount of rainfall during the development of first spring growth. These factors account for the inability of scab to propagate and develop rapidly. Judging from all the evidence, wherever there is a dry season little or no scab occurs, irrespective of other factors.

Citrus canker is not as serious a disease in South Africa as in the Gulf Coast States. Evidently the low mean temperature and small amount of precipitation during the growing season inhibits the rapid development of canker. At the most, the mean temperature for

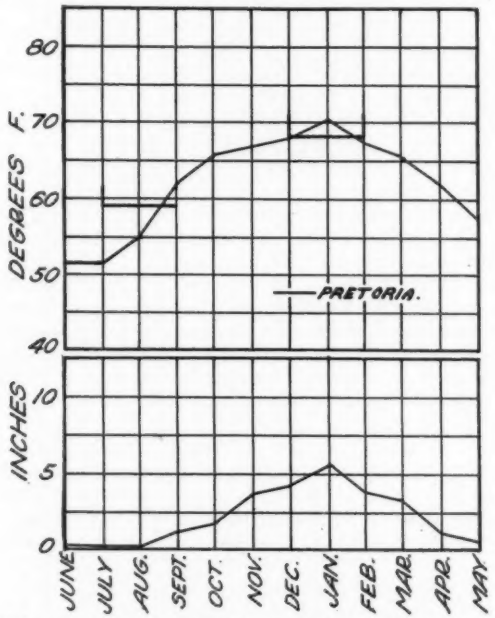

FIG. 5.—Climatic data for Pretoria, South Africa, and their relation to citrus scab and citrus canker

only three months are above the temperatures suitable for minimum infection (68° F.) by canker. The rainfall is rather low for these months, even though the mean monthly precipitation is at its maximum. In seasons when the temperatures and especially the precipitation are above normal, both scab and canker would be more active and destructive.

TYPES OF WEATHER FAVORABLE TO THE DEVELOPMENT OF CANKER BUT INHIBITING SCAB

Many varieties of citrus fruits are raised in the Philippine Islands and North Australia, but only in a small way. In these localities canker has been introduced within the last few years from Japan, but so far as is known to the writer no scab has ever been found.

In Siam and Indo-China, pummelos are grown extensively, as well as many varieties of citrus fruits common to China. While canker has been reported to be fairly well distributed, no data is at hand to determine how long the disease has been prevalent in these localities.

Evidently scab is not present in Siam and Indo-China, as Reinking (*7*) does not mention this trouble in his citrus-disease survey of those countries.

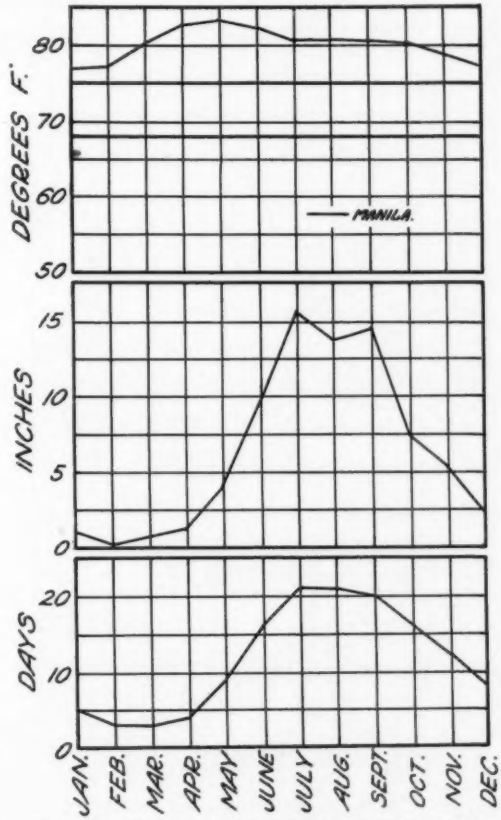

FIG. 6.—Climatic data for Manila, P. I., and their relation to citrus scab and citrus canker

Similarly, while canker has been reported from Borneo and Java, it is not known how long canker has prevailed there. No reports of the presence of citrus scab had been made from these islands.

The reason why scab does not thrive in the Philippine Islands has been pointed out in a previous publication (*5*). It is, in short, that the mean monthly temperatures throughout the year are above the maximum temperature (75° F.) at which successful infection can take place (fig. 6). It was also stated that the dry season might inhibit the carrying over of the disease from one season to another

The temperature and precipitation records for Darwin, Australia, are exactly comparable to those for Manila, P. I., so scab could not develop at this point.

Weather conditions somewhat similar to those which prevail at Manila, P. I., and Darwin, Australia, also prevails in Siam, Indo-China, Java, and Borneo, which accounts for the absence of citrus scab in those localities.

In Victoria and New South Wales, Australia, where scab is present as a disease of citrus, lower mean temperatures and sufficient precipitation during the first spring growth are at hand for infection. As yet no canker has been reported from these districts, but once introduced it undoubtedly would be quite serious.

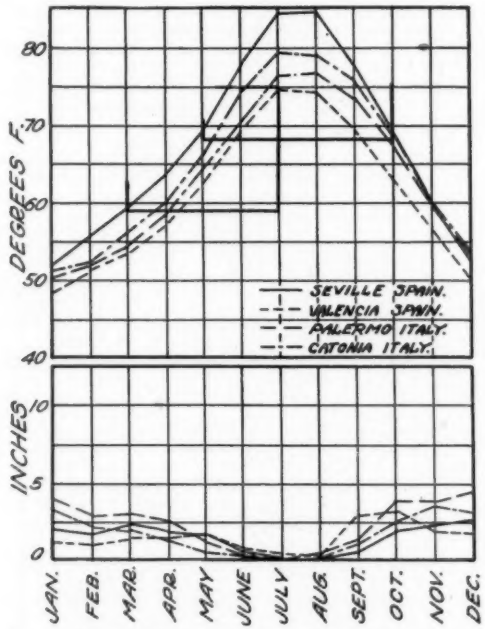

FIG. 7.—Climatic data for two points in Italy and three points in Spain, and their relation to citrus scab and citrus canker

In the Philippine Islands, conditions essential for serious damage by canker prevail from June on through the growing season, and can be correlated directly with the beginning of the rainy season (fig. 6). With a mean temperature about 80° F. or above for several months, and at least some rain during fifteen or more days during each month, canker becomes quite destructive. At the same time, these conditions just enumerated favor the rapid development of the citrus plants, which makes them much more susceptible to canker.

At Darwin, Australia, conditions similar to those at Manila, P. I., prevail throughout the year. Here again canker would be serious only during the rainy season.

TYPES OF WEATHER INHIBITING THE DEVELOPMENT OF BOTH SCAB AND CANKER

Wherever canker and scab occur it has been seen that both the temperature and precipitation curves have been rounding, ascending curves; and in the case of canker especially, the points of highest temperature and precipitation during the growing season have invariably produced optimum conditions for infection.

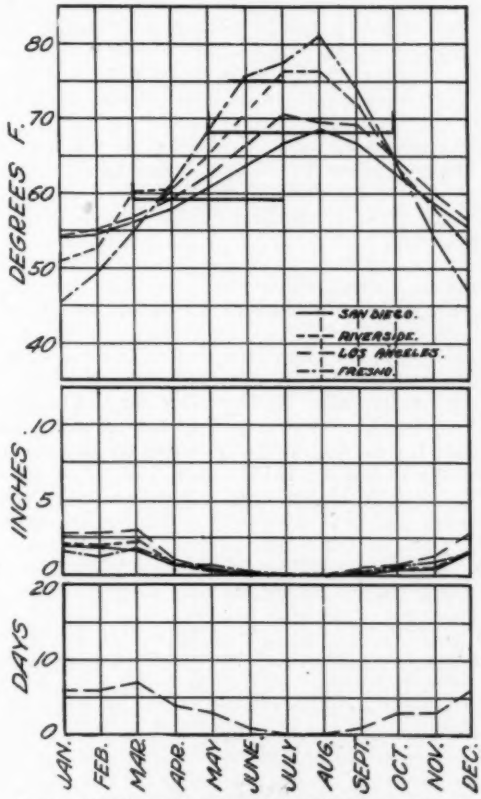

FIG. 8.—Climatic data for four points in California, and their relation to citrus scab and citrus canker

As has been stated, neither scab nor canker has ever been reported from the citrus-growing regions of California and the Mediterranean. In a previous publication (*5*) the writers have shown that scab could not develop or spread under California conditions, even though temperatures ideal for scab infection prevailed. The absence of precipitation during the development of the first spring growth is the inhibiting or limiting factor. A comparison of the temperature and precipitation at two points in Spain and Sicily (fig. 7) with the temperature and precipitation in California (fig. 8) shows that

here again, the lack of rainfall during the development of the first spring growth inhibits scab infection. The writers believe that this very largely explains the absence of scab in the Mediterranean countries and California.

In both California and at four points in the citrus regions of the Mediterranean, while the temperature curves are rounding and ascending as in other localities discussed, the rainfall curves are rounding but descending. In other words, during June, July, August, and September, months with optimum temperatures for canker infection, there are only two days in which rain normally falls at Los Angeles, Calif. The same conditions hold for the points in the Mediterranean countries to a somewhat less extent. At most points, less than 1 inch of rain per month falls during the four summer months.

In making comparisons of the influence of weather conditions on plants and their diseases in various parts of the world, weather records for specific time periods must be used rather than yearly means which are often misleading. The following illustration will serve as an example:

A rather serious outbreak of canker arising from infected nursery stock occurred at Laredo, Tex., in 1916 (fig. 9). It is generally assumed that semiarid conditions prevail in this vicinity, and that if canker developed under these conditions it could develop under southern California conditions also. If one compares the yearly precipitation and temperature near Laredo with the yearly precipitation and temperature of, say, Los Angeles, this assumption would seem reasonable. It is true that the monthly mean temperature was above the normal, and that there was a great deficiency of rain during the first half of 1916. However, from June on rainfall was quite abundant which not only stimulated the plants to a rapid growth but was sufficient to promote numerous infections. The mean monthly temperatures for July, August, and September were above 80° F., and this, together with the 3½ inches or more rain which fell during each of these months, provided favorable conditions for a canker outbreak.

In normal years canker would not be severe in this locality, but it could develop to some extent during the summer months when most of the rainfall occurs. While the normal yearly precipitation at Laredo is not much greater than that at Los Angeles, the greater part of it is distributed over the summer months and not the winter months as is the case in California.

CONCLUSIONS

Considering the temperature factor alone:

Citrus scab would be inhibited whenever a mean monthly temperature of 75° F. or above prevailed. In regions like the Philippine Islands, scab can not develop and spread at all, owing to the high mean temperatures prevailing throughout the year. In most of the citrus regions of the world, the period over which scab would be most active is limited to the spring season. In Japan, owing to the lower monthly mean temperatures, the period of scab activity is extended into July. At other points, as in California, temperatures suitable for scab development prevail but other factors essential for scab infection are lacking.

Citrus canker could develop in all of the citrus regions of the world sometime during the growing season. The period over which it would be active depends on the number of months having a mean temperature of 68° F. or above. The disease would be most severe at points having the greatest number of months with mean temperatures of 80° F. or above. Temperature is in no case a limiting factor for the development of canker in any of the citrus regions of

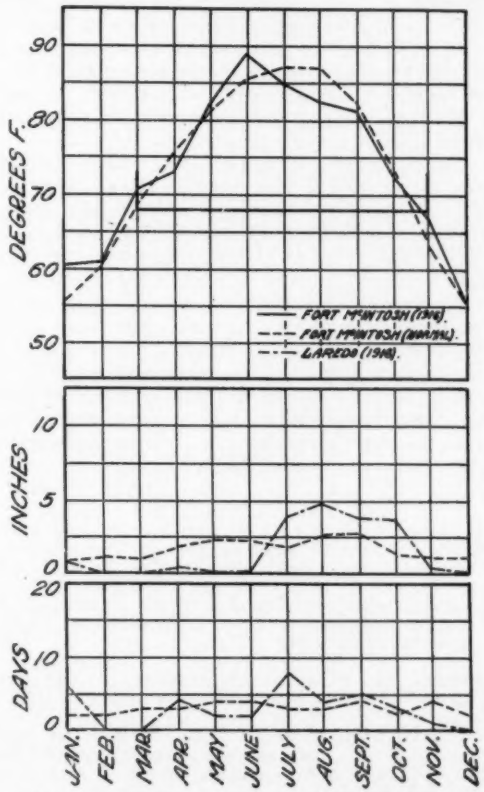

FIG. 9.—Climatic data for Laredo and Fort McIntosh, Tex., and their relation to citrus scab and citrus canker

the world. On the other hand, it is one of the limiting factors in scab development in that the maximum temperature for successful infection by scab is rather low.

Considering the moisture factor by itself:

Citrus scab is not a serious disease in any citrus-growing region which does not have an annual rainfall of at least 50 inches, rather evenly distributed over the year. Further, scab has never been reported for any citrus-growing region where a well-defined dry season occurs, especially when this dry season is coincident with high temperatures. Apparently, a dry season is a more important factor

in scab inhibition than a deficiency of precipitation during the spring months, as usually some precipitation occurs during the development of the first spring growth in most localities where scab is inhibited.

Likewise, a deficiency of precipitation during the growing season is the limiting factor in the development of citrus canker. A dry season does not inhibit the seasonal development of canker, for when the wet season is at hand canker again becomes active, as in the Philippine Islands.

Considering the combinations of environmental factors:

Whenever the temperature and precipitation curves are ascending and rounding curves, as they are for localities like the Gulf Coast States, China, and even South Africa, conditions essential for the development of canker are at hand, as these same conditions stimulate the rapid growth of the host plants, and thus make them more susceptible. On the other hand, no canker has ever been found in those localities, where, while the temperature curves are ascending and rounding ones, the precipitation curves are descending. In California, when the highest monthly mean temperatures are reached, the amount of precipitation is at its lowest point. If most of the annual rainfall occurred during the summer months rather than during the winter season, canker could develop to some extent, as conditions would then be similar to those prevailing in South Africa and at Laredo, Tex., in 1916. The amount, frequency, and seasonal distribution of precipitation is a limiting factor in the development of both diseases in question. Apparently the seasonal distribution is the most important factor to consider, for on the seasonal distribution of rainfall depends, to a large extent, the development or inhibition of these diseases in the citrus regions of the world.

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GENETICS OF MARQUIS × TURKEY WHEAT IN RESPECT TO BUNT RESISTANCE, WINTER HABIT, AND AWNLESSNESS[1]

By E. F. GAINES, *Cerealist, Washington Agricultural Experiment Station;* and H. P. SINGLETON, *Assistant in Crops and Soils, Irrigation Branch Station, Washington Agricultural Experiment Station*

INTRODUCTION

It is generally understood that varieties of wheat vary in their ability to resist attacks of bunt, *Tilletia tritici*, (Bjerk.) Wint. Three distinct types of resistance have been observed at the Washington Agricultural Experiment Station. The first type is that of the resistant winter wheat which, when spring sown, is immune; the second is that of the wheat which possesses the same relative resistance or susceptibility, whether fall or spring sown; the third and most remarkable type is that of the resistant spring wheat which, when fall sown, is quite susceptible.

INHERITANCE OF BUNT RESISTANCE

In order to clearly show the three types of resistance, Table I has been compiled from the records of experimental work on comparative resistance of American wheats, conducted at Pullman, Wash., in cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, of the United States Department of Agriculture. Part of this material has been published in United States Department of Agriculture Bulletin 1299 (*15*).[2]

TABLE I.—*Comparative bunt resistance of fall and spring sowings of several wheat varieties*

Name of variety	Washington No.	Year	Per cent of bunt		Type
			Fall	Spring	
Marquis	576	1919	74	10	3
Do	576	1921	77	7	3
Florence	634	1919	8	8	2
Bluestem	362	1919	90	92	2
Dicklow	630	1919	95	96	2
Early Baart	618	1919	76	87	2
Little Club	500	1919	96	97	2
Jenkin	526	1919	89	94	2
Red Chaff	421	1919	97	94	2
Hybrid 128	592	1921	94	(a)	
Winter Fife	371	1921	94	(a)	
Hybrid 143	590	1921	97	(a)	
Turkey	326	1921	10	0	1

[a] Did not head.

[1] Received for publication July 2, 1925; issued February, 1926. Revised from junior writer's thesis submitted for degree of master of science. The work has been done under the direction of the senior writer. The compilation of data and review of literature were done largely by the junior writer. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 121, College of Agriculture and Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 181.

Turkey represents the first type, which is resistant when fall sown but immune when spring sown. This variety is awned, and has the winter habit of development, i. e., if sown the latter part of April it will not head the following summer but remains a green grass clump, while Marquis heads and matures seed about the first of August.

With the exception of Marquis, all other varieties which matured from both fall and spring sowings represent the second type and possess practically the same relative amount of resistance regardless of time of sowing. Marquis alone represents the third type, which is resistant when spring sown but quite susceptible when fall sown. This variety is awnless and has the spring habit of growth. However, its susceptibility is not complete as is shown by the fact that it produced only 77 per cent of bunt the same season in which the three varieties, Hydrid 128, Winter Fife, and Hybrid 143, produced an average of 95 per cent. These three varieties have been considered as having practically no resistance (*6*). The difference, then, of 18 per cent in favor of Marquis may be taken as an indication that it is not completely susceptible but does possess some resistance. Similar evidence has been reported (*8*) in a cross between Hybrid 128 and Marquis. Of nine F_3 families comparatively low in amount of bunt produced, five produced the smallest percentage of bunt of the 13 rows in the F_4 generation. Of four very smutty F_3 families selected, one produced the highest percentage of bunt in the F_4 generation, and two produced more than the average of all F_4 rows in 1920. Five plants selected from the least smutty and five from the most smutty F_4 row produced an average of 78 and 91 per cent of bunt, respectively, in the F_5 generation in 1921. Since Hybrid 128 has no resistance, it seems that the difference was caused by a factor for resistance possessed by Marquis that is not affected by the winter resting period.

From previous studies (*7*, *8*) it seems that bunt resistance in some wheat varieties is not a simple Mendelian unit character, but that if Mendelian it is composed of multiple factors because a continuous series ranging from complete immunity to complete susceptibility was obtained. Apparently different wheats possess different kinds of resistance. Transgressive inheritance occurred in a cross between Turkey and Florence. From these two resistant wheats, segregates varying from immunity to 86 per cent bunt were obtained in the F_2 generation. Some of the immune segregates have bred true for immunity through nine generations (*9*).

Unlike many other parasitic fungi, bunt is not known to exist in more than one biologic form. Unless other forms are discovered, the problem of breeding for bunt resistance is much simpler than breeding for resistance to diseases which consist of several biologic forms, like the rusts, for example (*10*, *11*, *12*, *14*).

INHERITANCE OF WINTER HABIT

Cooper (*4*) reports finding the short vegetative period to be dominant over the long one in crosses between spring and winter wheats. Marquis, when crossed with three different winter wheats, produced approximately 13 spring plants to three winter in the F_2 generation. He explained this segregation on a two-factor basis in which one factor is dominant for winter and the other is an inhibitor of the

winter habit. The F_2 generation was satisfactorily analyzed on the foregoing assumption, but all three classes expected were not obtained in the F_3 generation, and two of the 13 F_3 families did not segregate according to any of the expected ratios. The small number of plants in the F_2 and the small number of families in the F_3, part of which were not explainable on his assumption, make it seem probable that other factors had a modifying influence.

In a cross between Marquis and Kanred, Aamodt (*1*) was unable to analyze the segregation on a definite factorial basis. However, he does conclude that the segregation for growth habit accords with Mendelian laws and that the results indicate multiple factors. The investigations of Vavilov and Kouznetsov (*16*) are similar to those of Aamodt. In the F_3 generation they found 9,551 spring plants to 2,191 winter plants, indicating the dominance of the spring form.

INHERITANCE OF AWNLESSNESS

In order to study the reaction of the Turkey parent in crosses with varieties other than Marquis, Table II was compiled from unpublished data obtained by the writers in other experiments.

TABLE II.—*Segregation of awnless and awned plants in the F_2 generation of crosses between Turkey and six other varieties of wheat*

Name of cross	Year	Number of plants awnless	Number of plants awned	Per cent awned
Fortyfold×Turkey	1919	423	175	29.3
Turkey×Triplet	1920	1,218	435	26.3
Triplet×Turkey	1920	1,101	380	25.7
Turkey×Ruddy	1920	999	306	23.4
Ruddy×Turkey	1920	1,163	405	25.8
Hybrid 128×Turkey	1920	1,343	473	26.0
Turkey×Winter Fife	1921	443	154	25.8
Winter Fife×Turkey	1921	423	133	23.9
Turkey×Jenkin	1921	1,155	370	24.3
Total		8,268	2,831	25.5

In all crosses of Turkey with the awnless varieties in Table II, the segregation approaches very closely a 3 : 1 ratio of awnless to awned plants. This is especially true when taking the totals of all crosses into consideration. A similar segregation in six other crosses has also been reported by the senior writer (*5*). The ratio of 3 : 1 proved to be 1 true awnless : 2 intermediate awnless : 1 true awned, by the behavior in the F_3 generation. Percival (*13*) has also found segregation in numerous crosses of beardless with bearded wheats to approach the 1 : 2 : 1 ratio. If the intermediate variants were classed with the awnless, the 3 : 1 ratio would be obtained. He further reports work of the Howards in which they obtained a segregation of 15 awned or partially awned to 1 quite awnless in a cross between an awned wheat and one which was quite awnless. They interpreted such a segregation as a dihybrid in which there was one factor for short awns and another for elongation for these short awns. However, in a cross in which Marquis is the awnless parent, the quite awnless types would not be expected, because Marquis has short awns from 0.3 to 1 cm. in length upon the upper fourth of the head.

In order to study the possibility of linkage between awns and bunt resistance, Table III was compiled from crosses of Turkey with five wheats other than Marquis which the writers had analyzed during the years 1918 to 1922.

TABLE III.—*Percentage of bunt produced by the awnless, mixed, and awned families in the F_3 generation of six crosses*

Name of cross	Year	Percentage of bunt		
		Awnless	Mixed	Awned
Turkey×Florence	1918	9.9	12.7	14.5
Hybrid 128×Turkey	1918	55.1	50.6	43.5
Fortyfold×Turkey	1920	46.1	45.3	51.0
Triplet×Turkey	1921	55.0	56.8	57.6
Turkey×Jenkin	1922	63.6	65.2	[a]
Turkey×Triplet	1922	57.9	59.4	[b]

[a] Only seed from awnless plants sown. [b] Only seed from awnless glabrous plants sown.

With the exception of Hybrid 128 × Turkey, it appears that there is some relationship between the presence of awns and increased susceptibility. However, in the exception, it is possible that the extra factor causing the extreme susceptibility of Hybrid 128 is linked with awnlessness, and in this case the awnless families of the F_3 generation would have a tendency to produce more bunt than the intermediate ones and these in turn would produce more than the awned. The apparent association of awns and susceptibility in the five other crosses may be due to one or the other of two things. It is possible that the presence of awns makes it easier to detect bunted plants, and that in the field separations part of the awnless bunted plants were counted as nonsmutted. The other is that the awned character of Turkey is actually linked with some susceptibility which is held in check by the chemotactic nature of Turkey resistance, and when the factors for resistance are eliminated in the recombination of chromosomes, the awned plants are more liable to become infected than the others. Regardless of the manner in which this variation was caused, the data indicate that if there is any linkage between the presence or absence of awns and relative amount of resistance, it is too small to interfere materially in selecting resistant plants of any type desired.

Aamodt (*1*) studied the possibility of linkage between awns and growth habit in his cross between Kanred and Marquis, but found no indication of it.

EXPERIMENTAL DATA

METHODS

The cross between Marquis and Turkey was made in 1918. The F_1 seed was planted that fall, and eight F_1 plants were harvested in 1919. The seed from five of these was sown in separate rows in the fall of 1919, and the F_2 families were harvested in 1920, one of which was selected as the progenitor of the F_3 generation. Approximately 150 seeds from each F_2 plant were infected by rolling the kernels in bunt until no more spores would adhere to them; then each packet was divided into two parts. One part (75 seeds from each plant) was sown in the fall, and the rest in the spring. Four replications of

each parent variety, and three of the F_2 sibs, were grown under the same conditions for comparison.

The F_2 generation was separated into awnless and awned plants, and notes were taken on the F_3 families as to whether the plants in each were all awnless, mixed, or all awned.

To obtain the amount of bunt produced by each row, the method described in 1920 (*7*) was used, which represents the approximate loss, and is comparable to the percentage obtained by a head count.

When fall sown, Marquis and Turkey head out and ripen at approximately the same date; but when spring sown Turkey does not even head unless sown very early. The fall-seeded F_3 rows headed and ripened uniformly, and all rows were pulled and records taken at one time. However, there was a distinct segregation in the ripening of the spring-sown material, and all plants were pulled and records taken as they ripened. The average date ripe was computed according to the formula $x=\frac{(bf)_1+(bf)_2+(bf)_3 \cdots}{N}$ in which x is the average date ripe, b is the date any given number of plants become ripe, f is the number of plants ripening on a given date, N is the sum of f_1, f_2, f_3, etc., which make up the total number of plants in the row.

In figuring the coefficient of correlation between any two sets of variants, the general rule of Babcock and Clausen (*2*) was modified by using class numbers as class centers. Class 1 was used as the assumed mean, and all corrections were made accordingly. Probable error was figured according to the formula $E=\frac{0.6745\ (1-r^2)}{N}$.

DESCRIPTION OF PARENTS AND F_1 GENERATION

Marquis (Wash. 576) is an awnless spring wheat producing hard red kernels. It is of hybrid origin, being a product of the Central Experimental Farm, Ottawa, Canada. The parents of Marquis are Hard Red Calcutta (an early ripening spring wheat, and Red Fife (a wheat which differs from Marquis in being taller and later, with kernels slightly longer and more pointed) (*3*).

Turkey (Wash. 326) is an awned winter wheat of the Crimean group. This strain was introduced from Kansas in 1906. It is very similar in taxonomic characteristics to other wheats of the Crimean group, but differs from some other strains in its resistance to bunt. Certain Crimean selections are bunt-free, while others produce as much as 75 per cent of bunt.

The F_1 seed of Marquis × Turkey was sown in the fall. The F_1 plants all ripened uniformly the following summer. They were all intermediate for awnlessness, having short awns on the upper florets. As the seed was not infected, no data on resistance to bunt were obtained.

DESCRIPTION AND ANALYSIS OF THE F_2 AND F_3 GENERATIONS

BUNT RESISTANCE

Four of the 160 families of the F_3 generation of Marquis × Turkey consisted of fewer than 10 plants in either the fall or spring sowing, and these were not considered. As was previously stated, three F_2 sibs and four rod rows of each parent were grown under the same

conditions as the F_3 generation. The data on bunt resistance of all material in both the fall and spring plantings are summarized in Table IV.

TABLE IV.—*Number of parent replications and F_2 and F_3 families of Marquis × Turkey falling into each class, when differences between class centers increase by an arithmetical progression of 2.5. This progression emphasizes the small differences of the resistant segregates*

Variety	When sown	Class center of bunt										Total
		0	0.5	3	8	15.5	25.5	38	53	70.5	90	
Marquis	Fall									3	1	4 replications.
	Spring				4							Do.
Turkey	Fall				4							Do.
	Spring	4										Do.
F_2	Fall								1	1	1	3 families.
	Spring			1	2							Do.
F_3	Fall			2	11	21	12	8	28	38	36	156 families.
	Spring	44	16	29	35	21	7	3	1			Do.

In breeding for bunt resistance, a given difference between two resistant segregates is much more significant than the same difference between two susceptible ones. For instance, a variety of wheat which produces an average of 1 per cent of bunt is evidently quite different from a variety which produces 10 per cent, but a variety which produces 70 per cent is not so different from one which produces 80 per cent. Therefore, a factor for resistance has a larger value when acting alone than when acting in cooperation with other resistance factors. As immunity is approached by the accumulation of factors, the value of the individual factor approaches 0, but acting alone it appears to have a value of about 20 per cent. In order to allow each factor its true value, whether acting alone or in combination with other similar factors, the use of an arithmetical progression seemed the more logical method to determine the size of the various classes. The F_3 segregates varied from immune to 100 per cent bunted, and were divided arbitrarily into 10 classes. All immune segregates were placed in the first class, this being 0. The rest of the population fell into nine classes, the last of which has a spread of 20 per cent. A progression of 2.5 between class centers placed the center of the second class at 0.5 per cent and the tenth at 90 per cent.

The resistance of the two parents is shown in Table IV. Marquis is fairly susceptible when fall sown, but does show a small amount of resistance in that three of the four replications were in the 70.5 per cent class. Marquis, when spring sown, falls into the 8 per cent class. Turkey, which falls into the 8 per cent class if fall sown, is immune in the spring sowing.

The F_2 generation was somewhat more resistant than Marquis in both sowings, but was not nearly so resistant as Turkey. However, in the F_3 generation, transgressive inheritance occurred and the spread in both the fall and spring sowings exceeded the extremes of both the parents. The resistance of Turkey does not account for those segregates falling into the 3 per cent class in the fall sowing, and there are too many immune segregates in the spring sowing for Turkey to be the sole contributor. The susceptibility of Marquis does not account for nearly all the segregates in the 90 per cent

class in the fall planting, or for those beyond the 8 per cent class in the spring planting.

The data in Table IV (shown graphically in fig. 1) bring out the similarity of the fall and spring sown F_3 families. In each curve there is a distinct division between the resistant and susceptible segregates. If it were possible to judge the relative resistance of the immune segregates in the spring planting, and the relative susceptibility of all the segregates in the 90 per cent class of the fall planting, each curve could be plotted so that it would drop to the base line at either end of the curve. If such an extension to both curves can be imagined, a striking similarity will be seen between the two. The susceptible part of each curve includes a larger part of the population. The difference in the bunt resistance of parents when fall and spring sown is evidently responsible for the difference in the location of the two curves, which indicates the probability of a similarity in the inheritance of resistance in both sowings.

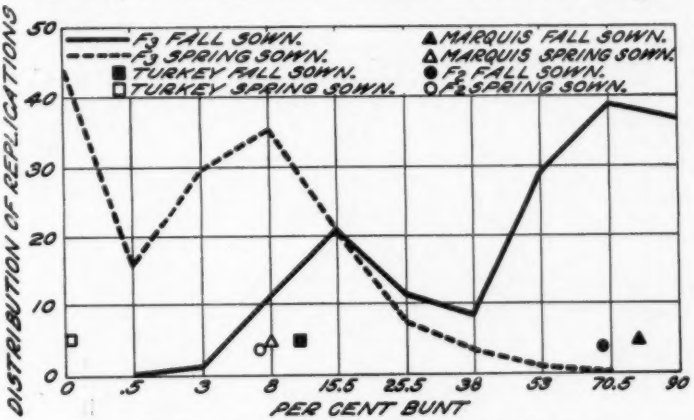

FIG. 1.—Bunt resistance of Marquis, Turkey, and their F_2 and F_3 segregates

In order to determine whether the factors producing resistance in the fall and spring sowings were the same, the coefficient of correlation between the two sowings was worked out as shown in Table V.

TABLE V.—*Correlation of 156 F_3 families of Marquis × Turkey, in respect to bunt resistance in fall and spring sowing*

Per cent of bunt in row in fall planting, subject. Per cent of bunt in row in spring planting, relative. Coefficient of correlation, 0.711 ± 0.027

Class centers	0	5	15	25	35	45	55	Fall-sown frequencies
5	10	2						12
15	19	2	1					22
25	9	2						11
35	1	4						5
45		8						8
55	3	18	1					22
65	1	15	4					20
75		13	7					20
85	1	9	6	4	2			22
95		3	6	3	1		1	14
Spring-sown frequencies	44	76	25	7	3		1	156

The high coefficient of correlation 0.711 ± 0.027 indicates that the varying degrees of resistance in either the fall or spring sowing is influenced by the same factors. The coefficient of correlation for the 88 families which produced no winter plants in the spring sowing was 0.708 ± 0.036, or practically the same as when all families were considered. The slight difference between the two coefficients is a significant indication of little or no linkage of bunt resistance and winter habit.

FALL-SOWN MATERIAL

From a study of the data already presented, it is evident that the factor for resistance which Marquis possesses plays an important part in the genetics of bunt resistance in this cross. In order to further study the effect of the Marquis factor, two crosses in which an entirely susceptible wheat was crossed with Turkey were compared with the Marquis × Turkey cross. These two wheats were Hybrid 128, which has practically no resistance, and Jenkin, which is similar to Hybrid 128 in this respect.

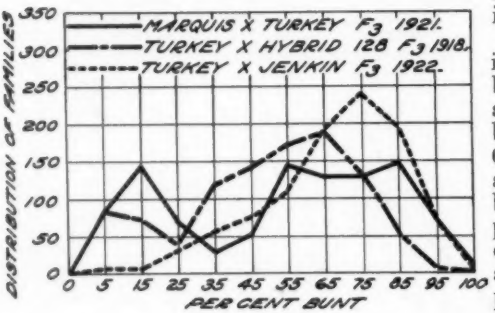

FIG. 2.—Bunt resistance of F_3 families of three crosses of Turkey with susceptible varieties

In 1918 three F_2 families of Turkey × Hybrid 128 produced an average of 51 per cent bunt, as compared to 0.6 per cent for Turkey and 63 per cent for Hybrid 128. The average per cent of bunt produced by the F_2 generation is more than that produced by Turkey, and less than that produced by Hybrid 128, but was within 10 per cent of the susceptible parent. Although 1921 was a much more favorable year for bunt production, the same relationship holds true for the cross between Marquis and Turkey.

The F_3 generation of Turkey × Hybrid 128 was grown also in 1918, while that of Turkey × Jenkin was grown in 1922. The distribution of the F_3 segregates in each of the foregoing crosses, together with that of Marquis × Turkey, are shown in Figure 2. Since the resistant segregates in each of the crosses are few, the distribution is shown by classes in which the class centers vary by 10. This spreads out the susceptible segregates more than if the class centers varied by an arithmetical progression.

There was considerable difference in bunt production of pure lines in 1918 and 1921. In 1918 the average bunt production of Turkey and Hybrid 128 was 0.6 and 63 per cent, respectively (*8*), while in 1921 it was 10 and 94 per cent (Table I). For this reason the Turkey × Hybrid 128 cross can not be compared directly with the one between Marquis and Turkey. However, 1921 and 1922 were very similar in that conditions were favorable for bunt production both years, and the F_3 of Turkey × Jenkin can be compared directly with the

F_3 of Marquis × Turkey. If the F_3 generation of Turkey × Hybrid 128 had been grown in 1921 or 1922 the bunt production of all segregates would have been increased, and a curve representing their distribution would be very similar to the one for Turkey × Jenkin.

The effect of the Marquis resistance working in combination with the Turkey resistance increases the number of resistant families and decreases the number of susceptible ones when compared to a cross in which the susceptible variety possesses no resistance.

Since transgressive inheritance occurred in Marquis × Turkey, it is evident that the two parents differ in their resistance. The assumption of one factor carried by each parent explains fairly well the distribution of the fall-sown F_3 families.

The resistance of Turkey will be called *a*, and that of Marquis *b*, in the discussion which follows. As susceptibility is partially (about three-fourths) dominant, the resistant factors are written as recessive. The genetic composition of the parents is AAbb for Marquis and aaBB for Turkey. The F_1 is AaBb, and the F_2 segregates in the manner shown in Table VI. Since the bunt production of AAbb, aaBB, and AaBb, is known by the performance of the parents and F_2 check rows, the remaining genotypes can be given a proportionate value. The value allowed for AABB was 95 per cent, this being the average between the extreme percentage of bunt produced by Marquis and the most susceptible F_3 segregate. The value allowed for aabb was 5 per cent, or the average between the most resistant check row of Turkey and the most resistant F_3 family. The values of the remaining four classes were computed from these five classes.

There are rather wide differences between the expected and actual numbers of families in each class when the nine genotypes are considered separately. However, the differences are greatly reduced when the classes are added according to the genotypes AA, Aa, and aa. This is significant because the *a* factor has about four times the value of the *b* (Marquis) factor in inhibiting the development of bunt when the parents are sown in the fall.

TABLE VI.—*Genotypes of the F_2 generation of fall-sown Marquis × Turkey as shown by performance of the F_3 families; also per cent of bunt expected from each genotype if each parent carries a different factor for resistance*

Genotype	Per cent bunt expected	Per cent population	Spread of class (per cent)	Number of families	
				Actual	Expected
AABB	95 (assumed)	6.25	93 to 100	7	9.75
AABb	90 [a]	12.50	84 to 92	24 46	19.50 39
AAbb	77 Marquis	6.25	76 to 83	15	9.75
AaBB	74 [b]	12.50	72 to 75	6	19.50
AaBb	69 (F_1)	25.00	65 to 71	18 61	39.00 78
Aabb	59 [c]	12.50	36 to 64	37	19.50
aaBB	10 Turkey	6.25	8 to 35	14	9.75
aaBb	9 [d]	12.50	8 to 35	29 49	19.50 39
aabb	5 (assumed)	6.25	0 to 7	6	9.75

[a] 95−77=18. 75 per cent ×18=13. 77+13=90.
[b] 95−10=85. 75 per cent ×85=64. 64+10=74.
[c] 77− 5=72. 75 per cent ×72=54. 54+ 5=59.
[d] 10− 5= 5. 75 per cent × 5= 4. 4+ 5= 9.

The value of a factor in heterozygous condition is $\frac{69}{95}$ (or 75 per cent) of one in homozygous condition.

The seasonal fluctuations of *a* and *b* explain the difficulty of classifying each segregate, and accounts for the differences found. Fluctuations greater than 20 per cent occurred in the Marquis check rows and in the F_2 sibs. It is logical to expect that similar fluctuations might occur in the F_3 segregates.

The foregoing statements bring out some of the difficulties encountered in attempting to interpret the inheritance of bunt resistance on a factorial basis. Seasonal and individual fluctuations of standard varieties are so great that several years' data on a large population would have to be taken into consideration in order to prove such an interpretation satisfactorily. It fits fairly well the small population of the F_3 generation grown in 1921. It also suggests that resistance reacts as a dihybrid in the cross between Marquis and Turkey.

SPRING-SOWN MATERIAL

The date of sowing in the spring was so early that some of the Turkey plants headed and ripened late in the summer. These plants were all immune. The resistance of spring-sown Marquis was high, the bunt production being but 8.5 per cent. The F_2 generation produced 7.2 per cent bunt, while the 156 F_3 families produced an average of 6.7 per cent as compared with 54.4 per cent in the fall-sown division. Since the coefficient of correlation between the fall and spring sown material was so high, each individual family produced considerably less bunt in the spring planting. However, this is to be expected because both parents show the same thing.

Transgressive inheritance was more noticeable in the spring-sown material. Thirty-one of the F_3 families were more susceptible than the most susceptible Marquis check row. One of these produced 55 per cent bunt or five times as much as spring-sown Marquis has ever produced in the writers' tests at Pullman.

It seems probable that the factors influencing the resistance of the spring-planted material are the same as those influencing the fall-sown, since the coefficient of correlation between the bunt production of the two plantings is high. (0.711 ± 0.027, Table V.)

In both parents resistance appears to be partially dominant in the spring sowing, but in order to prevent confusion, the genetic composition is written the same as for the fall sowing, i. e., Marquis AAbb and Turkey aaBB. The segregation of the F_2 generation as shown by the F_3 performance is given in Table VII. The value of AABB was assumed to be 40 per cent, which is approximately the average of the two most susceptible classes. As there were only four families in these two classes, it was assumed that they were the same genetically. The value for each of the remaining genotypes was taken or computed from the bunt records of the parents and the F_2 sibs.

TABLE VII.—*Genotypes of the F_3 generation of spring-sown Marquis × Turkey as shown by performance of the F_3 families; also per cent of bunt expected from each genotype if each parent carries a different factor for resistance*

Genotype	Per cent bunt expected	Per cent population	Spread of class (per cent)	Number of families	
				Actual	Expected
AABB	40 (assumed)	6.25	32 to 55	4	9.75
AABb	14 [a]	12.50	12 to 31	22	19.50
AAbb	8 (Marquis)	6.25	3 to 11 [b]	9	9.75
AaBB	8 [c]	12.50	3 to 11 [b]	18	19.50
AaBb	7 (F_2)	25.00	3 to 11 [b]	36	39.00
Aabb	1.6 [d]	12.50	0.1 to 2	23	19.50
aaBB	0 (Turkey)	6.25	0	11	9.75
aaBb	0 [e]	12.50	0	22	19.50
aabb	0 [f]	6.25	0	11	9.75

[a] 40−8=32. 20 per cent×32=6. 6+8=14.
[b] Since one of the F_3 segregates produced only 3 per cent bunt, the F_3 families producing this amount or more are not included in class Aabb.
[c] 40−0=40. 20 per cent×40=8. 8+0=8.
[d] 8−0=8. 20 per cent×8=1.6. 1.6+0=1.6.
[e] Probably "more immune" than Turkey.
[f] Probably "more immune" than aaBb.

The value of each factor in heterozygous condition is $\frac{1}{10}$ (or practically 20 per cent) of the homozygous condition.

On account of the genotypes AAbb, Aabb, and AaBb having approximately the same value, they could not be distinguished and were taken all together. However, each was allowed its proportionate part of the population falling into the general class expected from the three. The genotypes aaBB, aaBb, and aabb, were handled in the same way.

Considering that the expected class centers were close together, and that the fluctuations in bunt production of Marquis and the F_2 sibs were 3 and 7 per cent respectively, it seems that the dihybrid assumption accounts for the distribution of the spring-sown F_3 families even better than it does for these same families when fall sown.

WINTER HABIT

The F_1 and F_2 generations of Marquis × Turkey were grown from fall sowing. The F_3 families were grown in duplicate, and half of the seed of each F_2 plant was sown in the fall and the other half in the spring. Three F_2 sibs and four check rows of each parent were grown under conditions comparable with both sowings of the F_3 generation.

The spring sowing was done so early (April 1), that some of the Turkey plants headed and ripened. This fact makes a definite separation of the winter and spring plants difficult. If this planting had been two weeks later the Turkey would not have headed and the segregation would have been unmistable.

The fall-sown material ripened uniformly, and no data were taken regarding any small differences in the date of ripening. However, marked differences were noticed in the spring-sown material, and the ripe plants were pulled and recorded weekly. The first date of ripening was August 2, and the last was August 29. Those plants which were still green or had not headed at this late date were recorded as winter. The numbers of plants of the parents, F_2 sibs, and F_3 generation which were ripe on each date are shown in Table VIII.

TABLE VIII.—*Number of plants ripening weekly—of Marquis, Turkey, the F_2 sibs, and the F_3 families*

Description	Number of plants ripe each date					Number of winter plants
	Aug. 2	Aug. 9	Aug. 16	Aug. 23	Aug. 29	
Marquis	22	89	89	2	0	0
Turkey	0	0	0	0	24	92
F_2	32	78	117	16	4	4
F_3	262	1,263	3,763	498	365	456

The similarities and differences in time of ripening are shown, on a percentage basis, in Figure 3. There is no overlapping of the parents, and their extremes are obtained in both generations.

The F_2 and F_3 generations are similar in the percentages of plants ripe on each date. The Marquis plants were practically all ripe by August 16, while those of Turkey were all in the August 29 and winter classes. The curve of the F_2 and F_3 generation is not midway between the curve of Marquis and Turkey, but is well toward that of Marquis, indicating partial dominance of the spring habit.

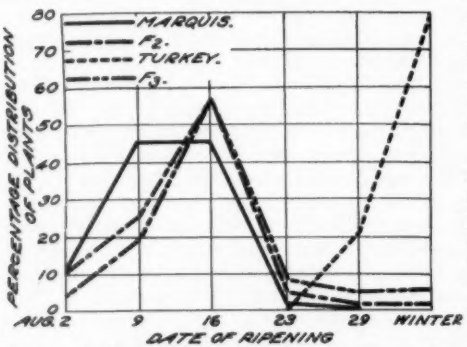

FIG. 3.—Distribution of plants ripening weekly, of Marquis, Turkey, and the F_2 and F_3 generations

On account of the early date of planting, it was impossible to separate the plants with certainty into winter and spring types, and any attempt to analyze the data on a factorial basis is therefore subject to criticism. In all probability most of the plants which ripened as late as August 29 were true winter. On this basis, the proportion of spring to winter plants was 35 : 1 for the F_2, and 7 : 1 for the F_3. This increase in winter plants in the F_3 generation may be due to either or both of the following reasons: (1) the winter factors, being recessive, breed true, and the percentage of homozygous segregates expected in the F_3 is greater than that expected in the F_2; (2) the F_2, which was the progenitor of the F_3, was grown from fall sowing and a larger proportion of the true spring segregates may have winterkilled, so these would not appear in the F_3 generation, and the relatively small numbers of F_3 plants ripening August 2 support this supposition.

An early ripening family and a late-ripening family of F_3 were selected as the progenitors of F_4 families. Five F_4 families were grown from the seed of each F_3 family. The seed from the early-ripening F_3 family produced only spring plants. These plants ripened about three days later than Marquis. The seed from the late-ripening F_3 family produced a majority of winter plants. Two

of the five rows produced no spring plants. From these and foregoing data it is evident that the inheritance of winter habit is controlled by multiple factors, and that the date of ripening is influenced by the cumulative effect of these factors.

RELATIONSHIP OF WINTER HABIT AND RESISTANCE

Turkey, as a rule, does not head when spring sown. However, in 1921 about 20 per cent of the plants headed and ripened due to early sowing. These plants were all immune. Evidently the resistance which permitted but 10 per cent of bunt in the fall sowing was able to prevent all bunt from appearing in the spring-sown material. If there is any linkage between winter habit and resistance, the later ripening plants of the F_3 generation should be more resistant than the earlier ones.

The coefficient of correlation between average date ripe and percentage of bunt in the F_3 families was 0.242 ± 0.68. This shows a slight relationship between early ripening and quantity of bunt produced, but the heterozygous condition of the majority of the families largely masks or lessens the real correlation.

Lateness of ripening due solely to environmental causes might inhibit or retard bunt production. If this were true, the later-maturing plants of Marquis should be freer from bunt than those maturing earliest. This is not the case, but it is rather the reverse, as is shown in Table IX. The F_3, however, shows a decided tendency toward increased resistance in the later-maturing dates. With the exception of the first ripening date, the F_2 shows greater resistance in the later-ripening plants. The earliest-ripening F_2 plants were very resistant, but the population was small. When the plants ripening on the first two dates are added, the total bunt produced is 8 per cent. This is a third higher than that produced on the later dates.

TABLE IX.—*Percentage of bunt produced by Marquis, and the F_2 and F_3 generations of Marquis × Turkey, on each of the five dates of ripening*

Variety	Date	Plants bunt free		Plants all bunt		Plants partly bunt					Total plants (number)	Total bunt (per cent)
								Heads				
		Number	Per cent	Number	Per cent	Number	Per cent	Wheat (number)	Bunt (number)	Per cent bunt		
Marquis	Aug. 2	17	77.3	0	0	5	22.7	51	16	23.9	22	5.4
	Aug. 9	60	67.4	0	0	29	32.6	197	92	31.8	89	10.4
	Aug. 16	60	67.4	0	0	29	32.6	309	110	26.3	89	8.6
	Aug. 23	2	100.0	0	0	0	0	0	0	0	2	0
	Aug. 29	0	0	0	0	0	0	0	0	0	0	0
F_2	Aug. 2	29	90.6	0	0	3	9.4	32	14	30.4	32	2.9
	Aug. 9	53	67.9	0	0	25	32.1	222	102	31.5	78	10.1
	Aug. 16	151	84.4	2	1.1	26	14.5	291	157	35.0	179	6.2
	Aug. 23	14	87.5	0	0	2	12.5	10	6	37.5	16	4.7
	Aug. 29	4	100.0	0	0	0	0	0	0	0	4	0
F_3	Aug. 2	181	69.1	4	1.5	77	29.4	645	467	42.0	262	13.8
	Aug. 9	913	72.3	16	1.3	334	26.4	2,665	1,983	42.4	1,263	12.5
	Aug. 16	3,002	82.2	22	.6	649	17.2	6,626	3,010	31.2	3,763	6.0
	Aug. 23	445	89.4	1	.2	52	10.4	457	268	37.0	498	4.0
	Aug. 29	331	90.7	1	.3	33	9.0	237	161	40.5	365	3.8

Since there was no tendency for the later plants of Marquis to be more resistant than the early ones, and this tendency was pronounced in both the F_2 and F_3 generations, it is evident that either there is some linkage between winter habit and bunt resistance, or that the cumulative effect of the factors for winter habit which cause difference in date of ripening also have an inhibiting effect upon bunt production.

Twenty-two of the 44 immune F_3 families produced no winter plants, and 17 ripened at least two weeks earlier than Turkey. If winter habit were completely linked with the AA resistance, all the immune segregates should resemble Turkey in date of ripening as well as in bunt resistance. This is impossible, however, if the assumption is correct that Turkey resistance is caused by a single factor and winter habit is caused by multiple factors. The winter-habit factors segregate as shown by the occurrence of spring types that mature later than Marquis. Linkage between resistance and one of the winter-habit factors would account for the increased resistance of the later maturing F_3 plants. The simplest interpretation, however, seems to be that there is little or no linkage between winter habit and bunt resistance, but that the factors for winter habit also cause increased resistance to a limited extent by means of some inhibiting influence.

AWNLESSNESS

The F_1 plants of Marquis × Turkey were all intermediate for awnlessness, having short awns on the upper florets of the head. The F_2 rows produced both awnless and awned plants. The number and per cent of plants in each class for each family is shown in Table X.

TABLE X.—*Number and percentage of awnless and awned plants in five F_2 families of Marquis × Turkey*

Row No.	Number of plants			Per cent population	
	Awnless	Awned	Total	Awnless	Awned
402901	162	47	209	77.5	22.5
403001	144	60	204	70.6	29.4
403101	95	66	161	59.0	41.0
403201	127	38	165	77.0	23.0
403301	175	72	247	70.9	29.1
Total	703	283	986	71.3	28.7

In making the separation in Table X, only the fully awned plants were considered awned. Those which were entirely awnless or which were somewhat intermediate were recorded as awnless. The number of plants in each family is fairly large, and the variation from the expected 3 : 1 ratio is rather wide.

The F_2 row (403201), which most nearly approached the 3 : 1 ratio, was selected as the progenitor of the F_3 generation. On account of the small number of seeds produced by some of the awned plants, the seed from five of these was not sown. The 33 awned F_2 plants which were saved produced only awned segregates in the F_3. The

awnless F_2 plants produced 35 homozygous awnless families, and 92 heterozygous ones. The genetic segregation of the F_2 family, as shown by the performance of the F_3, was 35 awnless : 92 heterozygous : 33(+5) awned. The expected monohydrid ratio of the same population is 41 : 83 : 41. The fact that the awned recessives all bred true makes the unit-factor hypothesis the most plausible. This explanation is in accord with results of other crosses in which Turkey was used as the awned parent (Table II), and is in harmony with Percival's interpretation (*13*).

LINKAGE OF AWNS AND RESISTANCE

There were four F_3 families which had fewer than 10 plants in either the fall or spring sowing, and since these were not considered in studying the inheritance of resistance they are disregarded. The number of awnless, heterozygous, and awned families, and the average per cent of bunt produced in each sowing, are shown in Table XI.

TABLE XI.—*Number of awnless, heterozygous, and awned F_3 families of Marquis × Turkey and percentage of bunt produced by each genotype in fall and spring sowings*

Genotype	Number of rows	Per cent bunt from	
		Fall sown	Spring sown
Awnless	34	67.8	10.0
Heterozygous	90	49.4	6.2
Awned	32	54.2	4.5

If only the awnless and awned genotypes are considered, it appears that there is some linkage between awns and the *a* factor for bunt resistance. However, the F_2 plants heterozygous for awns when fall sown produced less bunt than the awned, which is not to be expected if there is linkage. The heterozygous spring-sown families produced an intermediate amount of bunt which would be expected in case of linkage. The fact that 8 of the 44 immune families were awnless, 9 awned, and 27 heterozygous, indicates that there was little if any linkage between awns and the *a* factor for resistance.

LINKAGE OF AWNS AND WINTER HABIT

Seventy-one of the F_3 families produced one or more plants of winter habit of growth. Awnless, mixed, and awned families occurred in almost exactly the same proportion as in the pure spring group (14 : 41 : 16 compared with 21 : 51 : 17). If the awned character were connected in any way with winter habit, the group with winter plants should have a larger proportion of awned families, because Turkey carried both awns and winter habit. The foregoing ratio does show a slight tendency toward an increase in awning such as might be expected if the gene for awns were carried in one of three chromosomes carrying factors which delay the ripening period. If such were the case and there were no crossing over, it would be impossible for an awned family to ripen as early as Marquis. This is what actually happened, as shown in Table XII.

TABLE XII.—*Number of awnless, heterozygous, and awned F_3 families throwing winter plants; also average date ripe of each genotype*

Genotype	Number of families			Mean date ripe		
	True spring	Not true spring	Total	Earliest family	Latest family	Average all families
Awnless	21	14	35	Aug. 8	Aug. 27	Aug. 16
Heterozygous	51	41	92	do	Aug. 29	Do.
Awned	17	16	33	Aug. 13	Aug. 26	Aug. 17

The earliest awned row ripened five days later than the earliest awnless row. This might be mere coincidence, as the average of all awned families is but one day later than that of the awnless rows. The number of families is scarcely adequate for determining ratios where three factors are involved.

SUMMARY AND CONCLUSIONS

Marquis, when spring sown, is highly resistant to bunt; but when fall sown, it is comparatively susceptible. However, the resistance is not entirely inhibited by the winter resting period.

Turkey, when fall sown, is highly resistant; and when spring sown, is immune.

Transgressive inheritance occurred in both the fall and spring sowings of the F_3 Marquis $\times$ Turkey. Evidently the resistance of the parents is caused by different factors.

A correlation coefficient of 0.711 ± 0.027 was obtained between the amount of bunt produced by the fall-sown and tne spring-sown F_3 families. This indicates that the factors which cause resistance in the fall sowing are also operative in the spring sowing.

Resistance in the cross between Marquis and Turkey is apparently caused by two factors, the one carried by Turkey being much more "prepotent" than the one carried by Marquis.

The ratio of spring to winter plants was 35 : 1 in the F_2 population, and 7 : 1 in the F_3. This not only indicates that partial dominance of spring habit, but also that the inheritance of winter habit is controlled by multiple factors.

The later-ripening spring plants of the F_2 and F_3 generations were more resistent than the early ones.

Although the ratio of awnless to awned plants in the F_2 was only 2.5 : 1, all awned plants bred true in the F_3, indicating that awns are inherited as a unit character.

The awned F_3 families were somewhat more resistant than the awnless ones. However, the fact that only 9 of the 44 immune spring families were awned while 8 of them were awnless, indicates that there is little if any linkage between awns and resistance.

The average date ripe of the awnless, intermediate, and awned F_3 families was approximately the same. The ratio of awnless to awned families in the group which produced one or more winter plants and in the true spring group was approximately the same. If there is any linkage between awns and winter habit, the number of families is too small to determine it.

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ON THE NATURE OF RESISTANCE TO CABBAGE YELLOWS[1]

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INTRODUCTION

The subject of disease resistance in plants has aroused much interest among investigators in the field of plant disease. The literature of this topic has already been so amply reviewed by Vavilov (*28*),[3] Butler (*3*), Walker (*30*), and others that repetition here is unnecessary. A survey of the cases studied leads one to recognize the wide range of specific cases of resistance which are involved, each probably requiring a different explanation. It is apparent, however, that in most cases of highly resistant hosts invasion by the parasite does occur, but the phenomena of parasitism or resistance are variable and are not explained in any simple manner.

In connection with an investigation of cabbage yellows, certain comparative studies of resistant and susceptible varieties were made, the results of which are presented in this paper. As already noted for such closely related diseases as cotton wilt (*21*), cowpea wilt (*21*) tomato wilt (*9*), flax wilt (*27*), and others, it soon became evident that invasion of resistant cabbage plants usually occurred. By some means such plants were able to withstand the attack of the parasite, while those of the susceptible variety usually succumbed, under certain environmental conditions. This problem has been approached by a comparative study of the anatomy, cell-sap acidity, and the effect of plant extracts upon the growth of *Fusarium conglutinans* (Wollenw.), the parasite causing cabbage yellows.

In a preliminary study of the nature of resistance to cabbage yellows a number of possible lines of investigation were considered. Although it seems unlikely that strictly morphological characteristics may account for the resistant quality, it was considered possible that they may have some effect. For this reason the first studies made were morphological comparisons of resistant and susceptible cabbage plants.

Observations made continually over a number of years have shown that there are no appreciable, consistent differences in the general appearance, color, vigor, etc., of resistant and susceptible plants.

Comparisons of the root systems of plants of the resistant and susceptible varieties were made. Two weeks after being transplanted to the field the root systems of a number of plants of a resistant variety and of a susceptible variety were examined. The soil was

[1] Received for publication Mar. 5, 1925; issued February, 1926.

[2] This work was done while the writer was a graduate student in the department of plant pathology, University of Wisconsin. He is indebted to Prof. L. R. Jones and Dr. J. C. Walker for many timely suggestions and criticisms during the progress of the investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 198.

washed away from the roots and measurements were made directly of the depth, horizontal spread, and number of roots on each plant. These observations indicate that in general there is no appreciable difference in the root systems of resistant and susceptible plants at the time when there is the greatest difference in their behavior toward the disease.

Since Tisdale (*27*) found that flax plants resistant to wilt may form a layer of suberized cells in advance of the penetrating fungal hyphae, it was thought that such a phenomenon might occur in cabbages resistant to yellow. If such a hypothesis were true, resistant cabbage plants might form a thicker layer of cork cells or might form them more quickly than plants of susceptible strains. Roots, stems, and petioles of resistant Hollander, resistant All Seasons, and susceptible strains of the same two varieties were wounded with a sterile scalpel. After different lengths of time portions of the parts wounded were fixed, embedded, sectioned, and stained with safranin and light green. The suberized walls of the cork cells take up the safranin rather heavily and stand out well from the cellulose walls, which take up the green. As the result of study of numerous sections, both stained and fresh, freehand, it was concluded that there is no significant difference in the rapidity with which cork cells are formed or the thickness of such layers resulting when plants of the resistant and susceptible varieties were wounded.

RELATION OF CABBAGE EXTRACTS TO THE GROWTH OF THE FUNGUS

Physiological or internal factors as causes of resistance have been noted in a variety of instances. These differences between resistant and susceptible plants may be in the nature of the cell membrane or in the cell contents. In the latter case Ward (*33*, *34*), Gibson (*11*), Marryat (*20*), Stakman (*25*, *26*), and Reed and Crabill (*24*), working on various rusts, have found that the fungal hyphae enter the stomata of resistant plants, but are unable to penetrate the mesophyll cells far enough to cause serious injury. Walker and Lindegren (*31*) have shown that in the case of onion smudge there is a close correlation between scale pigments and resistance, the white varieties being quite susceptible, while the red and yellow varieties were highly resistant. They found that a cold-water extract of colored scales was highly toxic to spores and mycelium of *Colletotrichum circinans*, the onion-smudge fungus, but that a similar extract from white onion scales had no deleterious effect on the fungus. Reynolds [4] reported that flax plants resistant to the Fusarium wilt disease contain sufficient quantities of a chemical substance to inhibit the growth of the causal fungus in pure culture.

In order to ascertain whether there is a substance or principle in the cell sap of resistant cabbage plants sufficient to inhibit or prevent the growth of *Fusarium conglutinans* in pure culture, extracts from the tissues of cabbage plants were made in various ways. The first method tried was that of expressing the juice of green plants. Resistant All Seasons plants which had been grown in naturally infested soil at 26° C. for 30 days, during which time they showed no evidence

[4] Reynolds, E. S., Unpublished manuscript, 1924.

of disease, were macerated by being ground in a meat chopper and the juice extracted through cheesecloth. The juice of plants of a susceptible All Seasons strain grown under the same conditions, except that they were kept in healthy soil, was also extracted. Drops of the plant juice were placed on clean slides and drops of distilled water placed on other slides. One slide with plant juice and one with distilled water were placed in a Petri dish containing moistened filter paper. This was done in order to insure uniform conditions for the different liquids. A loop from a water suspension of microconidia from a young potato-agar culture of *F. conglutinans* was placed in each of the drops on the slides. The Petri dishes were incubated at 25° C. for from 14 to 24 hours, after which they were examined.

The fungus used in all the experiments recorded in this paper was obtained in the following manner: Plants of a susceptible strain of cabbage were set in yellows-infested soil obtained from Racine, Wis., where the disease has been prevalent for a number of years. When the plants showed definite symptoms of disease a portion of the stem of one of the plants was sterilized in mercuric chloride (1–1,000) for five minutes, washed in sterile water, and transferred to a sterile plate containing potato agar. After two days mycelium grew out from the cut ends of the stem. A single spore isolation was made by the method described by Keitt (*19*) and sterilized soil was inoculated with a spore suspension of the fungus thus obtained. Young cabbage plants were set in this inoculated soil and characteristic yellows symptoms developed after 10 days. The fungus isolated from such diseased plants compares favorably with the description of Wollenweber (*36*) and Gilman (*12*).

The results from several experiments repeated as described above were quite variable. In the first place, the dark-green color of the extracted juice, with numerous chloroplasts, made it very difficult to see the spores or mycelium of the fungus. This trouble was aggravated somewhat by the rapid development of bacterial contamination. In general, there seemed to be some inhibition of germination of conidia and growth of mycelium in the juice of resistant plants as compared with that in the juice of susceptible plants and in distilled water. In many cases there was a pronounced swelling of the spores and short germ tubes in the juice of resistant plants. But the density of the plant juice and the bacterial contamination associated with these studies made the results unsatisfactory. If the juice were diluted sufficiently to permit accurate observation of the spores there was no inhibition in germination of spores or growth of the mycelium. This experiment was repeated several times with the resistant strains Wisconsin Hollander, Wisconsin All Seasons, and Wisconsin Brunswick plants, together with plants of susceptible strains of the same varieties, but the results were much the same as those just described.

A method tried repeatedly to rid the cultures of the bacterial contamination and at the same time get away from the troublesome chlorophyll, was as follows: The plant juice extracted in the usual way was filtered through a layer of sterile kaolin 1 cm. thick into sterile flasks. The liquid filtrate had only a faint greenish color. Drops of the sterile filtrate were placed on sterile slides under sterile conditions and conidia of the yellows fungus were placed in them. Checks were made of sterile distilled water at the same time. The

results of these tests were rather variable, but, in general, germination and growth were better in the plant extracts, both from resistant and susceptible plants, than in the distilled water.

Since satisfactory studies of the effect of fresh plant juices on the fungus could not be made without bacterial contamination, a different method was used. Equal amounts of juice of resistant and susceptible plants, obtained in the usual way, were added to tubes of potato agar and autoclaved at 15 pounds for 30 minutes. Plates were poured with the agar and plant juice mixture, along with plates of agar without the plant juice. These plates were inoculated with the yellows Fusarium and incubated at 25° C. for six days. The fungus made better growth on the agar plant juice mixture than on the agar alone.

The above experiment was repeated, except that Uschinsky's solution was used in 150 c. c. flasks instead of the agar. It was found that with the addition of plant extracts from both resistant and susceptible plants to the nutrient solution growth was better in the cultures containing the plant juice than in those with the solution alone. The fungal growth increased in proportion to the amount of plant extract added. This is in harmony with the results obtained by Reynolds [5] in his work with flax wilt.

The writer has demonstrated repeatedly that the hyphae of the yellows fungus invade the xylem vessels of the taproot and lower part of the stem of resistant cabbage plants, but for some reason the fungus does not penetrate far enough or in sufficient quantity to cause serious injury or death of the plants. It seemed possible that there might be some substance in the vascular bundles of such resistant plants that would inhibit growth of *Fusarium conglutinans*. With this idea in mind the following experiments were performed:

Resistant Wisconsin Hollander plants which had been grown in naturally infested soil for 30 days at a favorable temperature for the development of the disease and susceptible Hollander plants grown for the same length of time in healthy soil were severed under water at the soil line and attached to the water-pressure apparatus described by Johnson (*16*). By means of heavy pressure the cell sap was forced from the vascular elements through the cut ends of the leaf veins. Drops of this extract, which was a clear liquid, were placed on clean slides, as previously described, and spore germination tests made. The spores in the liquid from both the resistant and susceptible plants germinated better and the germ tubes grew much faster than those in distilled water. These tests were repeated with plants of other resistant and susceptible varieties, with the same results in every case. There was no apparent difference in germination in the sap expressed from resistant and susceptible plants, and in both alike the presence of the sap stimulated growth as compared with distilled water. If there was an inhibitive substance in the vessels of resistant plants it was not present in the expressed liquid in sufficient quantities to cause any appreciable effect on the yellows fungus.

As the methods just described yielded no positive results, other attempts were made to ascertain whether there is a substance present in the tissues of resistant cabbage plants in sufficient quantities to inhibit the germination of spores of *Fusarium conglutinans*. Re-

[5] Reynolds, E. S. Unpublished manuscript, 1924.

sistant and susceptible Hollander plants were used in the first test, the plants of the resistant strain having been tested for resistance by being grown in yellows-infested soil for 30 days previous to being used, while the susceptible plants were grown in healthy soil for an equivalent length of time. The plants were removed from the soil, washed, cut into small pieces, and then dried in an electric oven at 60° C. for 48 hours. The dried tissues were pulverized thoroughly and steeped in cold water for 24 hours, 5 c. c. of water being used with each gram of dried tissue. The liquid was separated from the plant tissue by squeezing through cheesecloth. Spore-germination tests were made in the extracts from both resistant and susceptible plants in the usual way. In some cases there seemed to be a very definite inhibition of spore germination and germ-tube growth in the extract from resistant plants, but usually the inhibition was just as marked in the extract from susceptible plants.

This method was used in several tests with the resistant and susceptible strains of cabbage. Plants were dried at temperatures of 40°, 50°, 60°, and 85° C., and extracts made of the tissues. As in the other trials, the results were not consistent and clear-cut. No consistent differences were found between the water extracts from resistant and susceptible plants in their effect upon spore germination. In this case, however, bacterial contamination was not so evident as in the freshly expressed juices.

RELATION OF HYDROGEN-ION CONCENTRATION TO THE FUNGUS

The relation of the cell sap of the host plant as a factor in resistance to certain parasites has been noted by Comes (*5*, *6*). Gardner and Kendrick (*10*) found a close correlation between the hydrogen-ion concentration of the tissues of tomato and their resistance to the fruit-spot disease caused by *Bacterium exitiosum*. But Jones, Giddings, and Lutman (*17*), Hawkins and Harvey (*15*), Vavilov (*28*), and others found no positive evidence of its importance as the cause of resistance. Because of the possibility of such a factor being of importance in resistance to cabbage yellows, a study of the hydrogen-ion relations of *Fusarium conglutinans* was made.

In these studies four culture media were used, three of them liquid and the other solid. They were Uschinsky's, Richard's, and Czapek's nutrient solutions and potato-dextrose agar. The methods used were similar with the nutrient solutions and varied only slightly with the potato agar. Richard's solution was made up as modified by Karrer and Webb (*18*), while Czapek's and Uschinsky's solutions were made up according to the usual formulae. For adjusting the hydrogen-ion concentration in media of alkaline reaction, N/5 NaOH was used, and N/5 HCl was used for varying the reactions on the acid side. From the titration data of Karrer and Webb (*18*) the estimated amounts of alkali and acid necessary to bring the solutions to the hydrogen-ion concentration desired and the amounts of water necessary to bring all the solutions to the same volume were obtained, except for Uschinsky's solution, for which tables were made by experimentation. The amounts of acid and alkali varied somewhat from those used by these workers, because the amounts of solution used and the concentration of the nutrients were slightly different.

Each of the solutions was made up normal strength and then diluted in such a way by the addition of water and acid or alkali that the solutions contained a concentration of the nutrients equivalent to two-thirds of the original strength. Sixty c. c. of normal-strength nutrient solution, together with the desired amount of water, as indicated in the tables, were put into 250 c. c. flasks, plugged, and sterilized at 12 pounds pressure for 30 minutes. The nutrient solutions and the acid and alkali were sterilized separately, it being known that sugars react with acid or alkali when heated under pressure. After cooling, the cultures were removed to a culture chamber and definite amounts of acid or alkali were added by means of sterile graduated pipettes. The final volume of each culture was 100 c. c. The solutions were allowed to stand for 24 hours, after

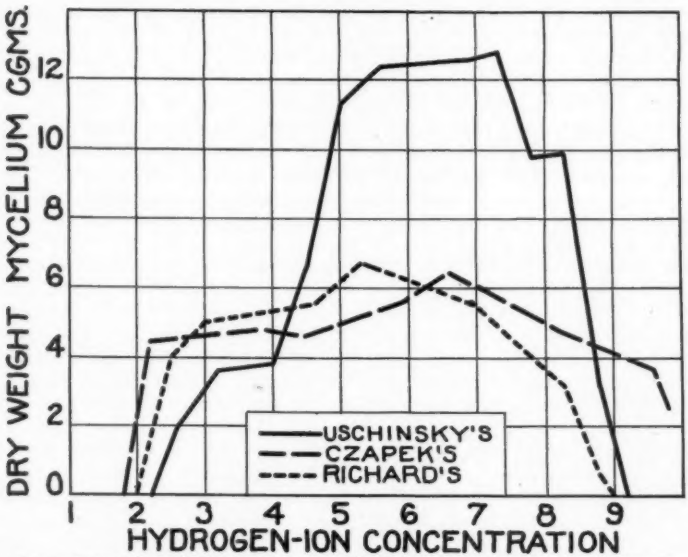

FIG. 1.—The relation of hydrogen-ion concentration to the growth of *Fusarium conglutinans* in liquid culture media

which the hydrogen-ion concentration was determined according to the colorimetric method of Clark and Lubs (*4*). The solutions were made up in lots of five at each concentration. One of them being used for obtaining the initial hydrogen-ion concentration, another was left uninoculated as a check, and three were inoculated with a small fragment of the fungus growing on potato agar. Care was taken to make the amounts of agar introduced into each flask as nearly equal as possible.

After being inoculated, the flasks, together with the uninoculated control flasks, were placed in an incubator, the temperature of which ran from 25° to 26° C. for 12 days. The mycelial mats were then removed from the flasks onto filter papers, previously dried and weighed, and the remainder of the mycelium filtered onto the paper by means of a Buchner funnel. The filter papers with the mycelial

mats were dried at 105° C. for 24 hours and reweighed. The titration data, together with the dry weights of the mycelium for each of the three nutrient solutions, are given in Table I. After the fungus was removed from the nutrient solution the hydrogen-ion concentration of it and the uninoculated check solutions was determined. These data are given in Table I. The fungous growth in the nutrient solutions is represented graphically in Figure 1.

TABLE I.—*Titration data for Uschinsky's, Richard's, and Czapek's solutions, and dry weights of mycelium of Fusarium conglutinans grown in these solutions*

USCHINSKY'S SOLUTION

No.	HCl	NaOH	Distilled H_2O	Total	Initial P_H	Final P_H of check	Final P_H of inoculated	Dry weight of mycelium
	C. c.	*C. c.*	*C. c.*	*C. c.*				*Gram.*
1	30 N/5		10.0	100	2.2	2.2	2.2	No growth.
2	26 N/5		14.0	100	2.6	2.6	2.8	0.0215
3	22 N/5		18.0	100	3.0	3.0	3.2	.0380
4	10 N/5		30.0	100	3.9	4.0	4.4	.0532
5	4 N/5		36.0	100	4.5	4.5	6.0	.0548
6	0 N/5		40.0	100	5.0	5.3	7.5	.1106
7		1 N/20	39.0	100	5.6	5.6	8.0	.1217
8		1 N/5	39.0	100	6.6	6.4	8.0	.1240
9		2 N/5	38.0	100	7.2	7.0	8.0	.1292
10		3 N/5	37.0	100	7.8	7.3	8.0	.0968
11		4 N/5	36.0	100	8.2	7.6	8.2	.0988
12		6 N/5	34.0	100	8.6	8.0	8.3	.0378
13		14 N/5	26.0	100	9.2	8.5	8.5	No growth.
14		15 N/5	25.0	100	9.4	8.6	8.6	Do.
15		16 N/5	24.0	100	9.6	8.6	8.6	Do.

RICHARD'S SOLUTION

1	6 N/5		34.0	100	2.0	2.0	2.0	No growth.
2	2 N/5		38.0	100	2.5	2.5	4.8	0.0414
3	0.5 N/5		39.5	100	3.0	3.0	5.0	.0533
4	0 N/5		40.0	100	4.5	4.4	5.4	.0560
5		0.7 N/5	39.3	100	5.2	5.2	5.4	.0637
6		1.0 N/5	39.0	100	7.0	8.8	5.5	.0526
7		1.5 N/5	38.5	100	7.5	7.3	6.0	.0448
8		4.0 N/5	36.0	100	8.4	7.8	6.4	.0323
9		6.0 N/5	34.0	100	8.8	8.0	7.2	.0137
10		10.0 N/5	30.0	100	9.0	8.5	8.4	No growth.

CZAPEK'S SOLUTION

1	7.0 N/5		33.0	100	1.9	1.9	1.9	No growth.
2	3.0 N/5		37.0	100	2.2	2.3	3.2	0.0420
3	0.5 N/20		39.5	100	3.7	3.7	4.7	.0430
4	0.0	0.0 N/5	40.0	100	4.4	4.4	4.7	.0416
5		1.0 N/5	39.0	100	6.5	8.3	5.3	.0632
6		2.0 N/5	38.0	100	8.1	7.6	6.6	.0610
7		9.0 N/5	31.0	100	9.6	9.2	7.8	.0372
8		16.0 N/5	24.0	100	9.6	9.6	9.6	.0230
9		18.0 N/5	22.0	100	9.6	(a)	(a)	(b)
10		20.0 N/5	20.0	100	9.6	(a)	(a)	No growth.

[a] No data taken. [b] Very slight growth.

It will be noted from Figure 1 that the maximum growth as indicated by dry weight of mycelium is at a different hydrogen-ion concentration with each of the three solutions. Growth took place over a wider range in Czapek's solution than in either of the others. The indicators used were not sensitive above P_H 9.6 on the alkaline side, and so the upper limits for growth could not be determined on this

medium. However, the fungus was definitely inhibited at about P_H 9 on the alkaline side and slightly above P_H 2 on the acid side of the scale with Richard's and Uschinsky's solutions. As in all the other experiments, the fungus made best growth in Uschinsky's fluid. This might be expected, because of the relatively large amounts of both nitrogenous and carbohydrate material in the solution. The fungus makes a very good growth on solutions containing both asparagin and ammonium lactate.

In order to show something of the hydrogen-ion changes produced in the medium by the fungus growing in it, the initial hydrogen-ion concentration of both the inoculated and uninoculated Richard's (A) and Uschinsky's (B) solutions are shown graphically in Figure 2. It will be apparent from an examination of this figure that slight changes took place on the alkaline side in the uninoculated solutions while standing for 10 days. This is more evident in Richard's solution than in Uschinsky's, while in the solutions in which *Fusarium conglutinans* grew the changes are much more noticeable. In Richard's solution the changes are quite striking. The fungus produced changes in the hydrogen-ion concentration of solutions ranging from high acidity to those of high alkalinity. The fungus tended to change the hydrogen-ion concentration of all the solutions to a concentration ranging from P_H 5.5 to P_H 6.6, the highest acid and alkaline cultures not being changed so much. But in Uschinsky's fluid the changes were quite different; in the first place, the changes on the acid side were very small as compared with those in Richard's solution, and the fungus seemed to have a tendency to change the hydrogen-ion concentration of the solutions toward the alkaline side.

In making up the agar cultures 75 c. c. of potato-dextrose agar made up double strength was placed in 250 c. c. flasks. The estimated amounts of acid or alkali necessary to bring the agar to the desired hydrogen-ion concentrations were obtained from titration tables previously prepared by experimentation. The amounts of distilled water necessary to make an equal volume in each flask, after the addition of the acid or alkali, were added, the flasks plugged, and sterilized at 12 pounds pressure for 30 minutes. The N/5 NaOH and N/5 HCl to be used in bringing about the hydrogen-ion range were sterilized separately. Immediately after being removed from the autoclave the flasks containing the agar were removed to a culture chamber and the estimated amounts of acid or alkali were added by means of sterile pipettes. The flasks were agitated to mix the solutions thoroughly with the agar, after which the agar was poured into sterile Petri dishes of uniform size, in such a way that the bottom of the dish was completely covered. In this way approximately equal amounts of agar were placed in each dish. There were three dishes at each hydrogen-ion concentration. When the plates had been poured the flasks containing the remainder of the agar still in liquid form were kept warm over a water bath until the actual hydrogen-ion concentration was determined. At the end of the experiment the agar in each plate was remelted and its hydrogen-ion concentration determined. The titration data are given in Table II.

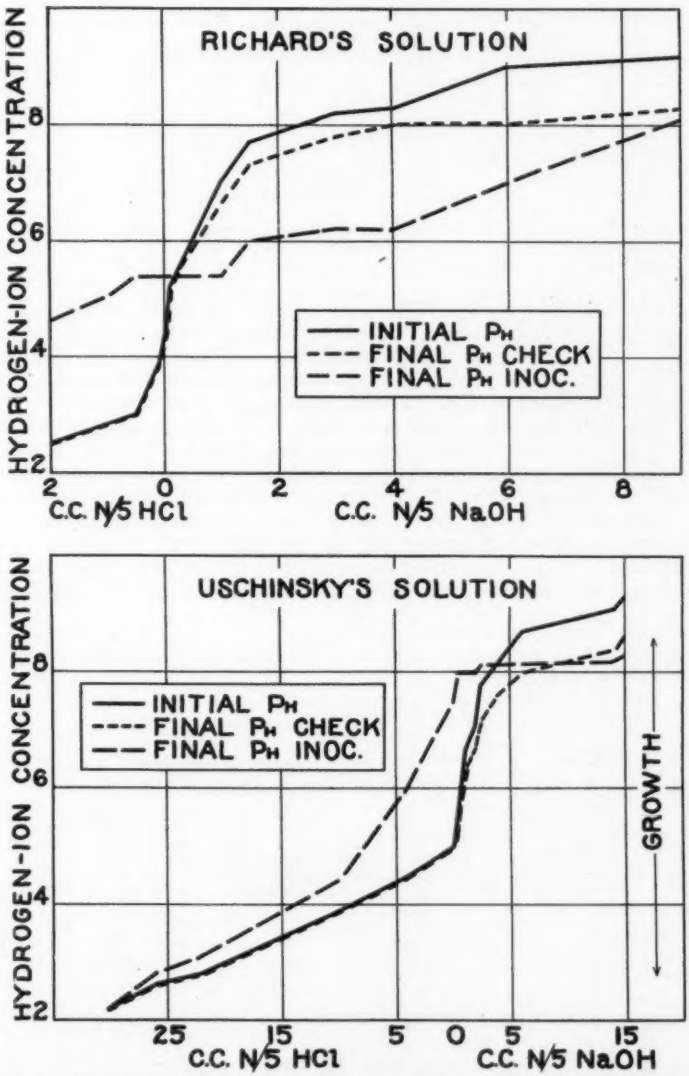

FIG. 2.—Changes brought about in the hydrogen-ion concentration of Richard's and Uschinsky's solutions inoculated with *Fusarium conglutinans* compared with those in the solutions uninoculated.

TABLE II.—*Titration data for experiment with potato agar*

Culture No.	Agar	N/5HCl	NaOH	Distilled H_2O added	Total	Initial P_H	Final P_H *
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*		
1	75	9.0		66.0	150	3.0	
2	75	7.5		67.5	150	3.3	
3	75	5.0		70.0	150	3.7	
4	75	2.0		73.0	150	4.4	
5	75	0.0		75.0	150	5.5	
6	75		3.0 N/5	72.0	150	6.6	
7	75		7.0 N/5	68.0	150	8.8	
8	75		14.0 N/5	61.0	150	9.5	
9	75		19.0 N/5	56.0	150	9.6	
10	75		5.5 N/1	69.5	150	+9.6	
11	75		8.0 N/1	67.0	150	+9.6	
12	75		10.0 N/1	65.0	150	+9.6	
13	75		15.0 N/1	60.0	150	+9.6	

* See Table III.

A small fragment of agar containing the mycelium of the yellows fungus was placed in each Petri dish. The cultures were incubated at 24° C. for seven days. Measurements were made of the diameter of the fungal colony at 24-hour intervals during the seven days. The data in Table III show that the fungus grows on potato-agar cultures with hydrogen-ion concentrations ranging from 3 to 9.6. The type of growth was quite different in the acid and alkaline cultures. In the acid cultures the mycelium grew quite flat, appressed closely to the agar, while in the alkaline cultures there was a decided development of aerial mycelium. The optimum development, as indicated by the diameter of the colony, was from P_H 4.4 to P_H 8, there being only slight differences in growth between these points The absolute limits for growth were not ascertained for this medium.

TABLE III.—*Effect of hydrogen-ion concentration on the growth of Fusarium conglutinans on potato-dextrose agar. Note the comparatively good growth at the high-acid and high-alkaline points*

Culture No.	Initial P_H	Diameter of colonies, in centimeters, after intervals of—						Final P_H
		2 days	3 days	4 days	5 days	6 days	7 days	
1	3.0	1.4	1.8	2.5	2.9	3.4	4.0	3.0
2	3.3	2.0	2.5	3.2	4.0	4.4	5.0	3.3
3	3.7	2.1	2.8	3.8	4.4	5.0	5.7	4.0
4	4.4	2.3	3.1	4.0	4.7	5.5	6.5	4.8
5	5.5	2.7	3.4	4.5	5.2	5.9	6.6	5.0
6	6.6	3.4	3.0	3.8	4.7	5.5	6.4	5.7
7	8.8	2.1	3.1	4.0	4.8	5.6	6.5	7.2
8	9.6	2.0	2.8	3.7	4.4	5.0	6.0	8.6
9*	+9.6	1.7	2.1	3.0	3.8	4.6	5.1	8.8
12	+9.6	1.0	1.8	2.4	2.8	3.3	4.2	9.6
13	+9.6	1.0	1.9	2.5	2.9	3.4	4.3	+9.6

* Cultures 9 to 13 were different only in having increasingly large amounts of alkali added, although the range of the indicators was limited to 9.6. The culture numbers correspond to those in Table II.

In the light of the results just described, it seemed possible that *Fusarium conglutinans*, when developing extensively in cabbage plants, might bring about changes in the hydrogen-ion concentration of the cell sap. Being aware of the fact that such investigations give little indication of what is taking place within the individual

cells occupied by mycelium of the fungus, the following experiment was performed: Four lots, each consisting of 15 plants, were used. The plants of lot I were resistant All Seasons and susceptible All Seasons in equal numbers, grown in healthy soil for 55 days, then in diseased soil at 21° to 30° C. for 30 days. These plants showed no symptoms of the yellows disease. Lot II consisted of plants of the same strains and grown under the same conditions as lot I, the only difference being that they were all severely diseased. Most of the leaves on every plant in lot II were distinctly yellowed, dwarfed, and twisted from the effects of the disease. The plants of lot III were of the resistant Hollander and susceptible Hollander strains grown under the same conditions as those of lots I and II. None of these plants showed any symptoms of disease. The Hollander plants of lot IV were grown under the same conditions as the other three lots, except they were transplanted into healthy soil at 12° to 14° C. for 30 days after being removed from diseased soil. These plants were badly diseased at the time of transplanting to healthy soil and during the last 30 days had changed very little.

The plants of the four lots were finely ground separately in a meat chopper and the juice extracted through cheesecloth. The liquids thus obtained were diluted to twenty times their volume with double-distilled water and the hydrogen-ion concentration was determined by the colorimetric method described by Duggar (7). The results given in Table IV would seem to indicate that *Fusarium conglutinans* brings about slight changes in the reaction of the tissues of diseased plants.

TABLE IV.—*Comparison of the hydrogen-ion concentration of the juice from healthy and diseased cabbage plants*

Lot	Strain	Condition of plants	Hydrogen-ion concentration
I	All seasons	Healthy	6.2
II	do	Diseased [a]	5.6
III	Hollander	Healthy	6.3
IV	do	Diseased [b]	5.9

[a] Plants collected at time of most severe attack of the disease.
[b] Plants kept at temperature too low for further disease development for 30 days after being removed from diseased soil in badly diseased condition.

It should be noted that such changes in the hydrogen-ion concentration of the juices of the diseased cabbage plants might have been a consequence of the ageing or senescence of the tissues quite apart from the effect of the particular causal agent. Such changes might occur on old or dying tissues of cabbage plants weakened by the black-leg or blackrot organisms.

The hydrogen-ion relations of the yellows fungus having been studied, the reaction of the tissues of resistant and susceptible plants were next considered. The cabbage plants used were grown in noninfested soil for 65 days at from 20° to 25° C. air temperature. The plants were about the size of those usually transplanted to the field in the southeastern Wisconsin cabbage sections. A lot of plants, 15 in number, roots, stems, and leaves together, of each variety, were collected at 9 o'clock in the morning, washed thoroughly, and ground

through a meat chopper. The juice was extracted through cheesecloth, after which it was diluted twenty times with double-distilled water. The hydrogen-ion concentration was then determined by Duggar's colorimetric method. This method was later checked by the hydrogen electrode and similar results were obtained. The strains tested were the resistant Wisconsin Hollander, Wisconsin All Seasons, and Wisconsin Brunswick, with susceptible strains of the same varieties. The plants used in the two tests varied somewhat in age and growing conditions. The results shown in Table V indicate that there are no significant differences in the P_H values of resistant and susceptible cabbage varieties.

TABLE V.—*Hydrogen-ion concentration of the expressed juice of various strains of cabbage*

Variety	Test No. 1	Test No. 2
Wisconsin Hollander	6.5	6.4
Commercial Hollander	6.3	6.3
Wisconsin All Seasons	6.4	6.4
Commercial All Seasons	6.3	6.2
Wisconsin Brunswick	6.2	6.4
Commercial Brunswick	6.3	6.5

It is to be noted in connection with the acid toleration of the yellows fungus that the hydrogen-ion concentration of the expressed juice from a cabbage plant may be quite different from that existing in the root cortex or xylem vessels of the plant in its normal state. It is likely that there may be differences in the acidity of different parts of the plant or in the same parts of the plant at different hours of the day and under different conditions. Harvey (*14*) found differences in the hydrogen-ion concentration of the juice from the petiole and midrib of a cabbage plant. It seems quite likely that the hyphae of the invading fungus may change the reaction of the host-cell contents surounding it, for in pure culture the yellow fungus tends to change the reaction of the medium from a high acidity to a lower or from high alkalinity to lower.

DISCUSSION

It has been shown herein that disease resistance to cabbage yellows is apparently not due to morphological characters of the host plant. There seem to be no significant, consistent differences in the root systems of resistant and susceptible plants. Although there is still the possibility that when some of the roots of resistant plants are injured or killed by the attacking fungus these plants are capable of putting out new roots faster or in greater numbers than susceptible plants. It has been noted in the course of certain experiments that plants of a resistant strain may be severely attacked by the yellows fungus, but often they remain in this condition for several weeks. Instead of dying, as do most of the susceptible plants, they put out new roots near the surface of the soil and may persist for some time in this condition.

The fact that the yellows fungus is frequently found invading the vascular elements of resistant plants well up into the stem, but apparently causing no ill effects in the plants, would seem to indicate

that strictly morphological characters could not account for the resistant quality. The difference between the condition in resistant and susceptible plants when grown in diseased soil seems to be the quantity of fungus in the tissues. The fungus can grow very well and produce microconidia within the vessels of suceptible plants, while fungal mycelium is produced quite sparingly in resistant plants, and the writer has not found the spores within the vessels of such plants.

The fact that *Fusarium conglutinans* is often found in the vessels of the stems of resistant plants showing no disease symptoms would indicate that the fungus is capable of invading the roots of such plants. This, together with the demonstration that resistant plants seem to form corky cells no faster or more numerous when wounded than do susceptible plants, would indicate that there must at least be some factor in addition to the formation of corky layers in advance of invading hyphae, if it occurs, which accounts for the resistant quality.

The writer has shown that differences in the hydrogen-ion concentration of resistant and susceptible plants do not seem to be sufficient to account for the resistance exhibited by certain strains of cabbage. *Fusarium conglutinans* grows well in pure culture over a wide range of hydrogen-ion concentrations on several artificial media. And the hydrogen-ion concentration of the expressed juice of resistant and susceptible cabbage plants comes well within the range for growth of the fungus. But it should be noted that this comparison gives little indication of the conditions actually existing in the vessels of plants attacked by the parasite when the hyphae are in contact with the cell contents. It is conceivable that the fungus mycelium growing in the vessels of resistant plants might increase the acidity of the cell sap sufficiently to actually inhibit its growth. It has been shown that the hydrogen-ion concentration of the juice extracted from diseased plants is somewhat lower than that of healthy plants of the same strain grown under the same conditions. This would seem to indicate that the fungus does bring about changes which increase the acidity of the liquids in diseased plants; but it seems likely that this increased acidity could not account for the inhibition of the fungus in resistant plants.

These factors probably being of little importance, it seems that there are two important possible explanations for the cause of resistant yet to be studied. As mentioned before, the first one of these to be investigated was the possibility of the existence of some substance or substances in the tissues of resistant plants which are toxic to the invading organism. Although this phase of the problem has been studied to some extent and no positive results obtained, the possibility that it is a partial explanation of the cause of resistance has not been eliminated. It was found to be impracticable to study the effect of the freshly extracted juices upon spore germination of *Fusarium conglutinans* because of the difficulty encountered in observing spores in the dark-green plant extracts and the density due to bacterial contamination, and when these extracts were filtered sufficiently to get rid of the chlorophyll, the inhibitive substances, if present before, were removed during the filtering process. Also, extracts from the tissues of resistant cabbage plants dried at temperatures ranging from 40° to 85° C. failed to at all consistently cause inhibition of germination or of germ-tube growth. But these results

do not convince the writer that there may not be a substance or substances present in the tissues of cabbage plants resistant to the yellows disease, which, if present in susceptible plants, are in much smaller quantities, that may be the cause or one of the causes for resistance.

Walker (*29*), Walker and Lindegren (*31*), and Walker, Lindegren, and Bachman (*32*) have noted that there are present in onion bulbs certain substances which are toxic to spores of *Colletotrichum circinans*. They divide these substances into two classes—(1) the volatile oils and certain nonvolatile compounds in the cell sap, and (2) the toxins associated with scale pigments. They find that the volatile compounds which arise from the succulent onion tissues are taken up by liquid suspensions of conidia and if present in sufficient quantity completely inhibit germination and retard growth. The toxicity of this volatile compound was found to be general to a number of fungi associated with diseases of the onion.

Reynolds [6] reports that there is present in the flax plant a cyanagenetic glucoside which produces HCN through hydrolysis by an enzyme. This glucoside is changed into HCN and glucose in the process of extracting. Reynolds concludes that the hydrocyanic acid in the extract is largely driven off in the autoclaving, while it remains in the extract which is filtered through a Mandler filter.

Hart and Peterson (*13*), in their investigations regarding the sulphur content and requirements of a number of crop plants, found that the cabbage plant contains much larger quantities of sulphur than had been suspected by previous workers. Peterson (*23*) mentions that volatile sulphur occurs in various plants of the Cruciferae family, especially the form existing in mustard oil, allyl-isothiocyanite, the chief volatile compound in black mustard (*Brassica nigra*). These mustard oils do not exist as such in the plants, but as glucosides, such as sinigrin in black mustard and sinalbin in white mustard (*Brassica alba*). That sinigrin contained sulphur was known to Berzelius (*1*) as early as 1840, also to Pelouze (*22*) and Dumas and Pelouze (*8*). Will and Körner (*36*) determined its composition and described a method for its preparation. A sinigrin-splitting enzyme discovered by Bussy (*2*) decomposes the glucoside into allyl-iso-thiocyanate, glucose, and acid-potassium-thiocyanate.

Peterson (*23*), while working with cabbage and rutabaga, found that when plants were dried at 95° C. about 25 per cent of the volatile sulphur could be retained and measured accurately. He also found that cabbage tissues contain 0.818 per cent sulphur on dry-weight basis, 9 per cent of which exists as volatile sulphur and 65 per cent as soluble sulphur.

It has been known for some time that sulphur, principally in the form of the higher polysulphides, is highly toxic to spores of various parasitic fungi. The volatile oil of the onion which Walker and his coworkers (*32*) found to be highly toxic to a number of fungi, is a sulphur-containing compound. It is suggested that there may be a sulphur-containing compound present in resistant cabbage plants in larger quantities than in susceptible plants, such a compound possibly existing in the form of a glucoside which is rapidly decomposed by an enzyme from the fresh extract of green plants or by the heat used in drying the tissues of such plants. This compound may be toxic to the invading hyphae of *Fusarium conglutinans*.

[6] Reynolds, E. S. Unpublished manuscript.

The nature of resistance to cabbage yellows is not clearly understood. The evidence accumulated indicates that morphological characters of the resistant plants are probably of little importance in this connection. The differences in hydrogen-ion concentration of resistant and susceptible plants seem to have little significance in explaining the cause of resistance. Studies of the comparative effects of various extracts of resistant and susceptible plants upon the yellows fungus have been made with not very satisfactory results. But the possibilities connected with such studies do not seem to have been exhausted. It is suggested that there may be a toxic principle in the tissues of resistant plants, possibly in the form of a sulphur-containing glucoside, which will account for the fact of resistance.

SUMMARY

Evidence has been accumulated which seems to justify the following statements in regard to resistance to cabbage yellows:

There seems to be no significant differences in root systems of resistant and susceptible plants.

Resistant cabbage plants do not form callous cells after wounding more rapidly or in thicker layers than do plants of the susceptible varieties.

It has been found that plants of resistant varieties are commonly invaded to a limited extent by the yellows fungus when growing in infested soil. This suggests the possibility of the existence of substances in the plant which are toxic to the hyphae of the invading fungus.

Attempts to observe the effect of expressed juice of cabbage plants upon the fungus were rendered uncertain because of difficulties associated with coloring matter and bacterial contamination.

The freshly extracted juice of cabbage plants added to nutrient solutions and autoclaved accelerates growth of the fungus in proportion to the amounts added, but there was no consistent difference in the effect produced by extracts from resistant and from susceptible plants.

Juice forced from vascular elements of cabbage plants by hydrostatic pressure accelerates spore germination and germ-tube growth of the fungus, but the liquids from susceptible and resistant plants gave similar results in this regard.

Water extracts from resistant plant tissues dried at from 40° to 80° C. have no consistent inhibitive effect upon the fungus which causes cabbage yellows.

The hydrogen-ion concentration of the expressed juice of different varieties is given. There are no differences in the hydrogen-ion concentration of the varieties tested sufficient to account for the resistant quality.

It is shown that *Fusarium conglutinans* grows over a hydrogen-ion range from P_H 3 to P_H 9 on the several media tried.

It appears that the resistant quality of certain strains or varieties of cabbage does not find its basis in morphological differences or in differences in cell-sap acidity. In all probability the true nature of yellows resistance is bound up with other factors.

Cabbage plants contain sulphur in appreciable quantities in the form of glucosides as well as volatile sulphur.

It is suggested that there may be a sulphur-containing compound present in cabbage plants resistant to the yellows disease in sufficient amounts to account in part, at least, for the resistance factor.

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